STUDIES ON THE CIRCULATION IN MAN.

VI. Observations on the Blood Flow in the Hands (Mainly) in Cases of Anemia.*

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Since anemia has a special influence on the blood flow in the peripheral parts, which it is essential to make clear, it has been judged well to begin the description of the results obtained in clinical cases illustrating various diseases with a discussion of the results in the anemias.

The common characteristic of the anemias as regards this point is a small flow in the hands as compared with the normal person of similar age and under similar external conditions. Thus in H. W., the flow was only 2.06 grams per 100 cubic centimeters of hand per minute for the right hand, and 1.82 grams for the left; *i. e.*, only one third to one sixth the amount to be expected in a healthy man.

H. W., a man aged 24 years, was admitted to Lakeside Hospital, suffering from pernicious anemia. Face and hands pale. Erythrocyte count 2,000,000,

Time.	Temperature of calorimeters.		Notes.	Time.	Temperature of calorimeters.		Notes.
	Right.	Left.			Right.	Left.	
3.08	30.16°	30.21°		3.21	30.20°	30.22°	Room temperature 23.6° C.
3.10	30.15°	30.19°	Room temperature 23.5° C.	3.23	30.21°	30.22°	
3.11	30.16°	30.20°		3.25	30.22°	30.22°	
3.12	30.17°	30.20°	Room temperature 23.4° C.	3.26	30.22°	30.22°	
3.13	30.17°	30.21°		3.27	30.23°	30.22°	
3.14	30.17°	30.21°	Ears pale	3.27½	30.23°	30.22°	
3.15	30.18°	30.22°		3.39	30.10°	30.09°	
3.16	30.19°	30.21°	Room temperature 23.6° C.	Cooling of calorimeters 0.13° in 11½ min. Volume of right hand 412 c.c., of left 378 c.c. Water equivalent of right calorimeter and contents 3.425, of left and contents 3.397.			
3.18	30.20°	30.22°					
3.19	30.19°	30.22°					

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hemoglobin 45 per cent. (April 16, 1911). Before death (May 2, 1911) the erythrocytes fell to 700,000. The hand flow was examined on April 19, 1911. Pulse (sitting) 120, and weak. Mouth temperature 37.35° C. The hands were put into the bath at 2.58 P.M., into calorimeters at 3.09 P.M., and taken out of the calorimeters at 3.27. 3,015 c.c. of water were in each calorimeter in all hand observations, unless otherwise stated.

Four months earlier the patient had an attack of erysipelas. The flow in the distal half of the right hand was 8.48 grams per 100 cubic centimeters of hand per minute, whereas in a healthy young man (M. C.) of the same age the flow in the distal half of the right hand was 20.77 grams. It has already been pointed out (1) that the flow in the distal part of the hand is decidedly greater than in the whole hand. Since a larger portion of M. C.'s hand was in the calorimeter in this experiment than of H. W.'s, the discrepancy is greater than appears at first sight. It is certain, therefore, that in H. W, in the experiment of December 17, 1910, the flow for the whole hand could not have exceeded four grams per 100 cubic centimeters per minute, notwithstanding the fever and the involvement of the arms in the inflammation.

December 17, 1910. At this time the anemia was well marked (erythrocytes 2,500,000), although less than on admission (November 30, 1910) when the count gave 816,000; hemoglobin 30 per cent. Rectal temperature 38.9° C. As he was too ill to sit up and the foot calorimeters had not yet been constructed, only the distal portion of his right hand was immersed in the bath and calorimeter, it being impossible to immerse the whole hand as he lay in bed. Pulse 112. Hand in bath at 3.18 P.M., in calorimeter 3.28½. 3,050 c.c. water in calorimeter. Room temperature 20.4° C. Hand out of calorimeter at 3.47.

Time.	Temperature of right calorimeter.	Time.	Temperature of right calorimeter.	Time.	Temperature of right calorimeter.
3.27½	28.92°	3.37	28.86°	3.44	29.00°
3.30	28.88°	3.39	28.91°	3.45	29.03°
3.34	28.86°	3.41	28.93°	3.46	29.08°
3.35	28.85°	3.42	28.96°	3.47	29.14°
				4.02	28.98°

Cooling of right 0.16° in 15 minutes. Volume of hand in calorimeter 175 c.c.

In another case of pernicious anemia (D. K.), a man forty-four years old, the flow on an extremely hot day with room temperature 29.1° C. rose to 8.35 grams per 100 cubic centimeters per minute for

the right hand, and 8.38 grams for the left, whereas in the normal young man, M. C., already referred to, the flows were respectively 17.98 grams and 16.65 grams with room temperature 27.1° C. In health the flow is not inferior at forty-four years to that at twenty-two. There is of course a cutaneous vasodilatation in response to the high external temperature, otherwise life would be endangered, yet the hand flow in D. K. remains markedly subnormal. It is perfectly intelligible that under these conditions the further reflex vasodilatation under the influence of warm water applied to the contralateral hand is feeble, the flow increasing only to 9.10 grams per 100 cubic centimeters per minute, while the reflex to cold is intense, the flow diminishing to 2.85 grams.

Time.	Temperature of calorimeters.		Notes.	Time.	Temperature of calorimeters.		Notes.
	Right.	Left.			Right.	Left.	
2.25½	31.52°	31.49°	Room temperature	2.36	32.02°	31.99°	
2.27	31.53°	31.52°	29° C.	2.37	32.09°	32.04°	Room temperature 29° C.
2.28	31.58°	31.55°		2.38	32.14°	32.10°	
2.29	31.62°	31.59°	Room temperature	2.39	32.20°	32.15°	
2.30	31.665°	31.63°	29° C.	2.40	32.27°	32.215°	
2.31	31.71°	31.695°		2.41	32.325°	32.27°	
2.32	31.78°	31.74°		2.42	32.39°	32.33°	
2.33	31.83°	31.81°	Room temperature	2.43	32.42°	32.37°	
2.34	31.895°	31.86°	29.1° C.	2.44	32.48°	32.425°	Room temperature 29.3° C.
2.35	31.96°	31.93°					
				2.45	32.52°	32.455°	At 2.45 left hand put in water at 8.1° C.
2.46	32.53°		He feels the water very cold	2.57	32.815°		
2.47	32.54°			2.58	32.85°		
2.48	32.555°		Still feels the water very cold	2.59	32.90°		
2.49	32.585°		The cold water is now at 9° C.	3.00	32.94°		
2.50	32.60°			3.01	32.995°		
2.51	32.62°			3.02	33.03°		
2.52	32.66°		Room temperature 29.2° C.	3.03	33.08°		Room temperature 29.2° C.
2.53	32.71°		He finds the cold water more	3.04	33.12°		
2.54	32.735°		tolerable	3.05	33.175°		Hand out of calorimeter at 3.05.
2.55	32.775°		At 2.55 left hand put in water at 43° C.	3.23	33.03°		Temperature of left calorimeter is now 32.22° C.
2.56	32.795°		Room temperature 29.2° C.				

Cooling of right calorimeter in 18 min. 0.145°, of left in 38 min. 0.235°C.

D. K., a blacksmith, 44 years old, was admitted to the City Hospital on July 1, 1912. He complained of shortness of breath, weakness, and buzzing in the ears. His skin had a yellowish cast. Marked anemia. Teeth good but pyorrhea present. There was a loud venous hum in the neck, especially loud on the left side in the sitting posture, and a soft blowing systolic murmur at the apex. Sec-

ond sound accentuated in pulmonary area. Paresthesia in both extremities (needles and ants). Ophthalmoscopic examination showed stippling of the retina and small miliary hemorrhages in both upper quadrants. X-ray examination negative. Blood examination: erythrocytes 1,648,000, leucocytes 5,400, hemoglobin 45 per cent. The erythrocytes showed marked variation in size; polychromatophilia; punctate basophilic cells; nucleated reds. Diagnosis: pernicious anemia. When the patient was last seen (August 21, 1912) there was no improvement.

July 2, 1912. Hands in bath at 2.14 P.M., and in calorimeters at 2.26½ P.M. Pulse 84 (sitting).

Volume of right hand 480 c.c., of left hand 476 c.c. He is right handed. Water equivalent of calorimeters and contents, right 3,479, left 3,476. Rectal temperature 38.2° C. Blood pressure: systolic 104, 59 (sound gone). Another observation: systolic 102, 61 (sound gone). At 82 the sound suddenly diminished, then increased and again suddenly diminished at 69, to disappear entirely at 61.

In a case of marked secondary anemia (S. K.) the flow was only 1.26 grams per 100 cubic centimeters for the right, and 1.18 grams for the left hand (for the last ten minutes in the calorimeters).

S. K., a woman, aged 48 years, was admitted to the City Hospital on August 26, 1912, with carcinoma of the uterus. She has had a foul smelling discharge from the uterus for a year, with pain in the right iliac region. She has lost greatly in weight. A cauliflower mass can be palpated on the cervix. The skin is pale and anemic. She has suffered from shortness of breath. Blood count on January 26, 1913: erythrocytes 2,112,000, hemoglobin 45 per cent. Died April 9, 1913. No metastases except microscopic ones in the liver.

January 24, 1913. Hands in bath at 3.06 P.M., in calorimeters at 3.16. Mouth temperature 37.15° C. Pulse (sitting) 108.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
3.15	31.32°	31.30°		3.30	31.20°	31.17°	23.4°
3.17	31.29°	31.26°	24.4°	3.31	31.195°	31.165°	23.3°
3.18	31.28°	31.25°	24.4°	3.32	31.19°	31.16°	
3.19	31.275°	31.25°		3.33	31.19°	31.155°	
3.20	31.27°	31.24°	24.4°	3.34	31.18°	31.15°	23°
3.21	31.27°	31.24°	23.9°	3.35	31.18°	31.15°	
3.22	31.26°	31.23°	23.2°	3.36	31.175°	31.14°	23°
3.23	31.25°	31.22°	23.1°	3.37	31.17°	31.135°	23°
3.24	31.24°	31.22°	23.2°	3.38	31.165°	31.13°	23.3°
3.25	31.23°	31.21°	23.3°	3.39	31.16°	31.12°	23.5°
3.26	31.23°	31.20°	23.4°	3.40	31.145°	31.11°	
3.27	31.22°	31.19°	23.5°	3.49	31.035°	31.00°	
3.28	31.21°	31.18°	23.4°				
3.29	31.21°	31.18°		Hands out of calorimeter at 3.40. Cooling of calorimeters 0.11° C. in 9 min.			

Volume of right hand 330 c.c., of left 324 c.c. Water equivalent of calorimeters with contents, right 3.359, left 3.354.

In a young man of twenty-two years (F. W.), the subject of hemophilia, the flow was 5.04 grams per 100 cubic centimeters per minute for the right hand and 3.94 grams for the left (for the last fifteen minutes in the calorimeters) with room temperature at 24.7° C. The flow was scarcely at all increased reflexly by the application of warm water to the contralateral hand, although it was easily decreased in the case of the right hand to 2.79 grams per 100 cubic centimeters per minute by the application of cold water to the left hand. At this time there had been no hemorrhage for six weeks.

Time.	Temperature of Calorimeters. Right.	Temperature of Calorimeters. Left.	Temperature of Room.	Notes.	Time.	Temperature of Calorimeters. Right.	Temperature of Calorimeters. Left.	Temperature of Room.
2.30	31.38°	31.34°			2.45	31.495°	31.40°	
2.31	31.36°	31.32°	23.2°		2.46	31.515°	31.425°	25°
2.32	31.37°	31.33°			2.48	31.57°	31.455°	25.2°
2.33	31.37°	31.335°	23.3°		2.49	31.59°	31.47°	24.9°
2.34	31.37°	31.335°			2.50	31.60°	31.48°	24.9°
2.35	31.375°	31.34°	23.1°		2.51	31.62°	31.485°	
2.36	31.38°	31.345°			2.52	31.635°	31.49°	24.7°
2.37	31.39°	31.35°	23°		2.53	31.65°	31.495°	
2.38	31.395°	31.355°	22.9°		2.54	31.68°	31.50°	24.4°
2.39	31.40°	31.355°			2.55	31.69°	31.515°	
2.40	31.40°	31.355°	22.5°	Closed window to raise room temperature	2.56	31.695°	31.52°	24.1°
2.41	31.40°	31.36°	22.6°		2.57	31.705°	31.54°	
2.42	31.41°	31.36°			2.58	31.715°		
2.43	31.425°	31.37°	22.7°		2.59	31.725°		24.5°
2.44	31.47°	31.385°			3.00	31.74°		
3.01	31.775°				3.12	31.935°		
3.02	31.795°		24.3°		3.13	31.94°		24.2°
3.03	31.805°				3.14	31.945°		
3.04	31.82°		24.1°		3.15	31.95°		24.3°
3.05	31.84°				3.16	31.945°		
3.06	31.88°		24.0°		3.17	31.945°		24.2°
3.07	31.895°				3.18	31.945°		24.1°
3.08	31.905°			At 3.08 left hand in water at 8° C.	3.19	31.945°		
					3.42	31.68°	31.10°	
3.09	31.92°			Left hand begins to ache				
3.10	31.925°			Left hand beginning to get red; it was pale in the warm water	Cooling of calorimeters, right 0.265° in 23 min., left 0.44° in 44 min.			
3.11	31.93°							

F. W., an engineer, aged 22 years, was admitted to the City Hospital on October 8, 1912. He stated that he had had rheumatism in the right leg for some time. Twelve days before admission he bumped it against a bench. It became

gradually red and swollen, with considerable pain, so that he could not walk. Then the leg became black and blue. It was opened, as it was thought that it might be suppurating, but only a large hematoma, perfectly sterile, was found. He stated that one brother died of bleeding after a tooth had been pulled. An uncle on his mother's side bled to death from the nose. He did not know of any hemophilic cousins. A grandfather and grandmother were cousins. At seven years of age he cut his hand and almost bled to death. After a cut on the foot he bled for several days. The gums and nose bleed occasionally for two or three days at a time, but his nose has not bled for five months. His leg became infected after it was opened and continued to discharge blood and serous liquid. It was treated by injections of horse serum and then of gelatin and gradually healed. On October 25 the blood examination showed the following: erythrocytes 2,376,000; leucocytes 7,200 (polymorphonuclears 61.4, eosinophils 1.6, small mononuclears 20.6, large mononuclears 9.1, transitionals 5.7). On October 31 the erythrocytes were 3,026,000. He was discharged on January 21, 1913, his leg being cured.

December 27, 1912. Hands in bath at 2.20 P.M., in calorimeters at 2.30½ P.M., taken out of calorimeters at 3.19. At 2.57 left hand put into water at 42.8° C.

Volume of right hand 449 c.c., of left 403 c.c. He is right handed. The left hand is noticeably less than the right. The left arm is also distinctly smaller than the right. He attributes this to rheumatism which used to affect the left wrist. Water equivalent of calorimeters with contents, right 3,454, left 3,417. Rectal temperature 37.25° C. Blood pressure: right arm, systolic 124, 73 (sound gone).

In Mrs. M. A., a woman thirty-eight years old, with an erythrocyte count of 3,000,000 and a hemoglobin percentage of 80, the flow was 4.70 grams per 100 cubic centimeters per minute in the right hand, and 5.44 grams in the left hand (for the last thirteen minutes in the calorimeters) with room temperature 23.1° C., a flow below the normal for her age. The tardiness with which the point was reached at which the thermometers began to rise uniformly, indicating stubborn vasoconstriction, illustrates a characteristic of this group of cases.

Mrs. M. A., aged 38 years, was admitted to the City Hospital on January 1, 1913. She has six children and has had no miscarriages. She menstruated last in August. She sometimes misses two or three months, but has never stopped so long before except when pregnant. She complains of pains in the right side about the liver. In July the abdomen became enlarged and the feet swelled. She denies that she has ever used alcohol. On admission she was jaundiced over the entire body. The abdomen was distended and fluid was present in it. Veins over abdomen are engorged. The liver extends to the umbilicus on the right side and three fingers' breadth below the costal margin on the left. The edge of the liver is hard and sharp but no nodules can be felt. The spleen is hard and easily palpable. X-ray examination negative. Wassermann negative. On January 13, 1913, the erythrocyte count was 3,004,000, the leucocyte

count 6,600, hemoglobin 90 per cent. On January 23 the erythrocytes were 2,500,000, hemoglobin 80 per cent., leucocytes 7,000 (polymorphonuclear 72 per cent., eosinophils 1 per cent., large mononuclears 9 per cent., small mononuclears 13 per cent., transitionals 4 per cent.). On January 28 the erythrocyte count was 3,600,000; hemoglobin 70 per cent. There was some hematuria all along (albumin in the urine and erythrocytes), also a few casts. Gynecological examination showed nothing abnormal. Examination of the thorax negative. Radial pulse, symmetrical, equal, regular, good volume and tension. No evidence of parasites. The blood flow in the hands was examined on January 28, 1913. On this date she states that she is stronger than when she entered the hospital. She has no pain now. Her lips are red, though the face is somewhat pale. She used to have good color. Her appetite is not very good, but she is not at all emaciated. Blood pressure: systolic 118, 85 (change in sound). Diagnosis: cirrhosis of liver. She was discharged improved on February 19, 1913.

Hands in bath at 2.33 P.M., in calorimeters at 2.45, and out of calorimeters at 3.09. Pulse (sitting) 80. Mouth temperature 36.85° C.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.44	31.12°	31.04°		2.59	31.115°	31.07°	23.1°
2.46	31.07°	31.02°	23.1°	3.00	31.125°	31.085°	
2.47	31.07°	31.02°		3.01	31.15°	31.11°	
2.48	31.08°	31.02°	23.15°	3.02	31.17°	31.135°	23.05°
2.49	31.09°	31.03°		3.03	31.18°	31.15°	
2.50	31.09°	31.03°	23.2°	3.04	31.195°	31.165°	23°
2.51	31.085°	31.035°		3.05	31.20°	31.185°	
2.52	31.09°	31.03°	23.2°	3.06	31.21°	31.21°	23.1°
2.53	31.09°	31.035°		3.07	31.22°	31.225°	
2.54	31.09°	31.035°	23.15°	3.08	31.23°	31.24°	23.2°
2.55	31.09°	31.04°		3.09	31.24°	31.25°	
2.56	31.095°	31.04°		3.19	31.115°	31.135°	
2.57	31.10°	31.05°	23.2°				
2.58	31.105°	31.055°		Cooling of calorimeters in 10 min., right 0.125°, left 0.115°. Volume of right hand 330 c.c., of left 332 c.c.			

In three cases of the chlorotic type the flow although probably below the normal was by no means scanty. Thus in E. D., a girl of sixteen years with an erythrocyte count of 4,100,000 and a hemoglobin percentage of 50, the flow in the right hand was 6.25 grams per 100 cubic centimeters per minute, and in the left 5.76 grams (for the last thirteen minutes in the calorimeters) with room temperature 22.2° C. The contralateral vasodilatation produced by warmth was slight; the contralateral vasoconstriction produced by cold was much more distinct.

E. D., a Hungarian girl, aged 16 years, a dispensary patient, has been ill for about six months suffering from shortness of breath on exertion, as in walking. Her appetite was very poor for about nine months, but is now better. For eleven months she worked eight hours a day in a cigar factory and to this she attributes her illness. She is pale, while before her illness she says she had a good color. A venous hum can be heard in the neck. Her hands are rather thin. She has occasional headaches with severe temporal pain. She states that other girls in the factory have become ill in the same way. On February 4, 1913, erythrocyte count 4,100,000, hemoglobin 50 per cent.

The flow in the hands was examined on February 5, 1913. Hands in bath at 2.30 P.M., in calorimeters at 2.40½ P.M. Pulse (sitting) 130, and weak. She had just walked up to the third floor room where the observations were made. Mouth temperature 37° C. At 3.09 P.M. left hand put into water at 43° C., at 3.23 P.M. into water at 8.5° C.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.39	31.08°	31.04°		2.55	31.105°	31.115°	
2.41	31.00°	31.07°		2.56	31.12°	31.12°	22°
2.42	31.00°	31.06°	21.5°	2.57	31.13°	31.135°	22.2°
2.43	31.01°	31.05°		2.58	31.17°	31.17°	22.2°
2.44	31.005°	31.04°	21.5°	2.59	31.195°	31.19°	
2.45	31.01°	31.04°		3.00	31.20°	31.20°	22.25°
2.46	31.01°	31.06°		3.01	31.215°	31.21°	
2.47	31.01°	31.07°	21.6°	3.02	31.23°	31.22°	22.2°
2.48	31.04°	31.075°	21.7°	3.03	31.26°	31.23°	
2.49	31.05°	31.08°		3.04	31.29°	31.24°	
2.50	31.065°	31.09°	21.9°	3.05	31.29°	31.25°	
2.51	31.07°	31.095°		3.06	31.295°	31.26°	22.3°
2.52	31.08°	31.10°	22.1°	3.07	31.30°	31.27°	
2.53	31.095°	31.105°		3.08	31.30°	31.265°	
2.54	31.10°	31.11°		3.09	31.305°	31.26°	22.5°

Time.	Temperature of		Time.	Temperature of		Time.	Temperature of	
	Right calorimeter.	Room.		Right calorimeter.	Room.		Right calorimeter.	Room.
3.10	31.31°		3.18	31.395°		3.26	31.49°	22.4°
3.11	31.32°		3.19	31.415°	22.45°	3.27	31.495°	
3.12	31.32°	22.5°	3.20	31.43°		3.28	31.495°	
3.13	31.32°		3.21	31.45°		3.29	31.495°	
3.14	31.325°	22.4°	3.22	31.465°	22.45°	3.30	31.505°	22.4°
3.15	31.34°	22.45°	3.23	31.48°		3.31	31.505°	
3.16	31.365°		3.24	31.48°		3.32	31.505°	
3.17	31.38°		3.25	31.48°	22.5°	3.33	31.54°	Hand out of calorimeters at 3.33.
						3.48	31.31°	Temperature of left at 3.48, 30.72° C.

Cooling of calorimeters, right 0.23° C. in fifteen minutes, left 0.54° C. in thirty-nine minutes. Volume of right hand 303 c.c., of left 275 c.c. Water equivalent of calorimeters with contents, right 3,337, left 3,315. Pulse (sitting) at the end of the

examination 96. It is stronger than with the more rapid rate before the examination. Blood pressure in left arm, systolic 105, 73 (sound gone), 85 (sound decreased).

In Mrs. A. P., who was still nursing completely her thirteen months old baby, the red cell count was 4,600,000, the hemoglobin 70 per cent., the flow in the right hand 8.75 grams per 100 cubic centimeters per minute, and in the left hand 8.09 grams with room temperature 23.5° C.

Mrs. A. P., aged 32 years, a dispensary patient, was admitted on February 3, 1913. The face, lips, and sclerotics are anemic. She suffers from shortness of breath on exertion. She has been ill for three weeks. She has had a good deal of trouble with her teeth. She is fairly fat and well nourished. Her feet get very cold up to the knees, but her hands do not get so cold. The physical examination revealed nothing special. On February 3 erythrocyte count 4,600,000, hemoglobin 70 per cent. On February 4 hemoglobin 70 per cent. by Haldane-Gower's hemoglobinometer. Her hands seem well supplied with blood, but it does not ooze very freely from a prick either in the finger or in the ear lobe.

The blood flow in the hands was examined on February 4, 1913. Pulse (sitting) 104, shortly after climbing to the third floor. Hands in bath at 2.22 P.M., into calorimeters at 2.39 P.M., and out of calorimeters at 2.53.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of calorimeters.		Notes.
	Right.	Left.			Right.	Left.	
2.38	30.88°	30.81°		2.48	31.14°	31.08°	Room temperature 23.45°.
2.40	30.90°	30.80°					
2.41	30.89°	30.82°	23.2°	2.49	31.16°	31.105°	
2.42	30.89°	30.82°		2.50	31.22°	31.14°	Room temperature 23.5°.
2.43	30.91°	30.84°					
2.44	30.955°	30.90°	23.4°	2.51	31.27°	31.175°	
2.45	31.01°	30.945°		52	31.29°	31.20°	
2.46	31.04°	30.99°	23.45°	.53	31.335°	31.22°	Room temperature 23.6°.
2.47	31.095°	31.035°		3.15	31.10°	30.97°	
				Cooling of calorimeters in 22 min., right 0.235°, left 0.25°. Volume of hands, right 390 c.c., left 386 c.c. Mouth temperature 37.0°. Pulse (sitting) at end 88.			

Water equivalent of calorimeters with contents, right 3,407, left 3,404. Blood pressure in left arm, systolic 115, 63 (sound gone). Another observation, 114, 62.

In Mrs. T., a woman aged thirty years, with an erythrocyte count of 4,584,000 and a hemoglobin percentage of only 40, the flow in the right hand was 6.48 grams per 100 cubic centimeters per minute, and in the left 7.20 grams, with room temperature 22.6° C.

Mrs. T., aged 30 years, height 5 feet 2 inches, dispensary patient. She has children. She has been treated for falling of the womb. Menses regular. Suffers from weakness, pains in the back, and headache. She easily becomes short of breath. She has not been losing flesh. On January 9, 1911, the erythrocyte count was 4,584,000, hemoglobin 40 per cent.

The blood flow in the hands was examined on January 11, 1911. Hands in bath at 3.30 P.M., in calorimeters at 3.40, and withdrawn from calorimeters at 3.55. 3,150 c.c. of water in each calorimeter.

Time.	Temperature of			Time.	Temperature of		
	Calorimeter.		Room.		Calorimeter.		Room.
	Right.	Left.			Right.	Left.	
3.39	30.36°	30.36°	22.6°	3.49	30.59°	30.56°	
3.42	30.43°	30.39°		3.50	30.62°	30.60°	
3.43	30.46°	30.40°		3.51	30.65°	30.62°	
3.44	30.48°	30.41°		3.52	30.68°	30.65°	
3.45	30.50°	30.43°		3.53	30.70°	30.68°	
3.46	30.50°	30.46°		3.54	30.71°	30.69°	
3.47	30.52°	30.48°		3.55	30.72°	30.70°	
3.48	30.56°	30.51°		4.14	30.52°	30.53°	23.2°

Cooling of calorimeters in nineteen minutes, right 0.20° C., left 0.17° C. Volume of right hand 275 c.c., of left hand 245 c.c. The left hand is visibly smaller than the right. She is right handed. Pulse (sitting) 72. Mouth temperature 37.6° C. Water equivalent of calorimeters with contents, right 3,450, left 3,426.

In a case of anemia (O. L.) secondary to copious gastric hemorrhage in a man aged thirty-six years, the erythrocyte count was 2,250,000, the hemoglobin percentage 45, the flow in the right hand 7.02 grams per 100 cubic centimeters per minute, and in the left 6.90 grams, with a room temperature of 25.4°. These blood flows are rather small, but very much greater than in H. W. and S. K., where the anemia is associated with much graver general changes than in O. L., whose blood changes are probably at this time conditioned mainly or entirely by the hemorrhage. The contralateral vasodilatation to warmth is good, the flow in the right hand being increased from 7.02 grams to 9.93 grams per 100 cubic centimeters per minute when the left hand is immersed in warm water after the usual preliminary diminution to 5.95 grams. The reaction to cold is fair.

O. L., a man, aged 36 years, height 5 feet, 7½ inches, weight 146 pounds. He is a molder, is married, and has one child. He has heavy work in a hot atmosphere. He has always had good health until now. On April 27, 1911, ten days before admission to the dispensary he vomited a large quantity of blood. Since

then he has suffered from weakness and shortness of breath, also headache and pain in the ears, relieved by lying down, and some epigastric tenderness. Erythrocyte count 2,250,000, hemoglobin 45 per cent. He entered Lakeside Hospital and reappeared at the dispensary on September 9, 1911. The blood count was now good (erythrocytes 6,000,000, hemoglobin 90 per cent., leucocytes 6,200). A test meal (September 28, 1911) showed free hydrochloric acid 30, fatty acid 54, lactic acid 0. In June, 1912, he was operated on at another hospital. He was told that an ulcer was found and that the stomach adhered to the liver. On May 3, 1913, in Lakeside Hospital an inoperable carcinoma was found at the cardia.

The blood flow in the hands was examined on April 27, 1911. Pulse (sitting) 96. Mouth temperature 37.3° C. Hands in bath at 3.30 P.M., and in calorimeters at 3.40½ P.M.

Time.	Temperature of calorimeters.		Time.	Temperature of right calorimeter.	Notes.	Time.	Temperature of right calorimeter.	Notes.
	Right.	Left.						
3.40	29.36°	29.29°	3.53½	29.99°	Room temperature 25.5°	4.06	30.68°	Room temperature 25.3° C.
3.42	29.39°	29.33°	3.54	30.01°				
3.43	29.42°	29.37°	3.55	30.09°		4.07	30.71°	
3.44	29.47°	29.41°	3.56	30.15°		4.08	30.735°	At 4.08 left hand dried and wrapped in towel.
3.45	29.51°	29.44°	3.57	30.23°	The warm water is now at 40°			
3.46	29.56°	29.50°						
3.47	29.61°	29.54°	3.58	30.31°				
3.48	29.68°	29.63°	3.59	30.38°	At 3.59 put left hand in water at 7.7°	4.09	30.77°	Room temperature 25.3° C.
3.49	29.73°	29.70°						
3.50	29.80°	29.74°				4.10	30.79°	
3.51	29.88°		4.00	30.42°	Left hand put into water at 43° C.	4.11	30.82°	
3.51½		29.82°				4.12	30.88°	
3.52½	29.92°					4.13	30.91°	
			4.01	30.46°	Room temperature 25.4° C.	4.14	30.98°	Hand out of calorimeter at 4.14.
			4.02	30.49°	Room temperature 25.35° C.	4.22	30.89°	At 4.22 temperature of left is 29.61°.
			4.03	30.52°				
			4.04	30.56°				
			4.05	30.61°				

Cooling of calorimeters, right 0.09° in 8 min., left 0.21° in 30½ min.
Volume of right hand 470 c.c., of left 448 c.c. Water equivalent of calorimeters with contents, right 3.471, left 3.453.

In a case convalescing from scurvy (J. B.), a man in the prime of life, the flow in the right hand was 5.53 grams per 100 cubic centimeters per minute, and in the left 6.30 grams, with room temperature 23.9° C. The vasomotor reflexes were good, the flow in the right hand increasing to 10.47 grams, after a preliminary dip to 2.54 grams for one minute, when the left was immersed in warm water and sinking to 5.66 grams for the first three minutes of immersion of the left hand in cold water. For the two minutes succeeding the first minute of immersion of the left hand in cold

water the flow in the right is only 4.68 grams per 100 cubic centimeters of hand per minute.

J. B., a laborer, aged 34 years, a patient at the City Hospital, was recovering from scurvy. There was a history of a very poor diet for a long time, bread and tea for some months with no vegetables or fresh meat. He slept under a saloon counter. There was bleeding from the gums, and his legs showed abundant signs of previous hemorrhages. On admission (March 13, 1912) erythrocyte count was 5,000,000, hemoglobin 60 per cent. March 15, 1912, hemoglobin 60 per cent.

The blood flow in the hands was measured on April 4, 1912. Pulse (sitting) 52. Mouth temperature 36.7° C. Hands in bath at 1.02 P.M., and in calorimeters at 1.15 P.M.

Time.	Temperature of calorimeters.		Time.	Temperature of right calorimeter.	Time.	Temperature of right calorimeter.	Notes.
	Right.	Left.					
1.14½	30.54°	30.53°	1.28½	30.795°	1.42	31.41°	
1.16[1]	30.51°	30.51°	1.30	30.84°	1.43	31.44°	
1.17	30.53°	30.53°	1.31	30.89°	1.44	31.50°	
1.18	30.545°	30.55°	1.32[1]	30.93°	1.45	31.52°	
1.19	30.56°	30.57°	1.33	30.99°	1.46	31.56°	
1.20	30.575°	30.605°	1.34	31.06°	1.47	31.595°	At 1.47 left hand dried and wrapped up.
1.21[1]	30.595°	30.625°	1.35	31.12°	1.48	31.62°	
1.23	30.66°	30.70°	1.36[3]	31.18°	1.49	31.63°	
1.24	30.70°	30.74°	1.37	31.21°	1.50	31.69°	
1.25	30.72°	30.79°	1.38	31.225°	1.51	31.74°	
1.26	30.75°	30.82°	1.39	31.24°	1.52	31.79°	At 1.52 hand out of calorimeter.
1.27[2]		30.84°	1.40	31.28°			
1.27½	30.79°		1.41	31.36°	2.04	31.63°	At 2.04 temperature of left is 30.46°.

Cooling of calorimeters: right 0.16° C. in twelve minutes, left 0.38° in thirty-seven minutes. Volume of right hand 407 c.c., of left 410 c.c. He said he was left handed, and the slight preponderance in volume of the left hand bore this out, although as he only spoke Slovak it was difficult to be quite sure. The left hand had more calluses than the right. Pulse (sitting) at end of examination 60. Rectal temperature 37.1° C. Water equivalent of calorimeters with contents, right 3,421, left 3,423.

Finally, in a case of severe nosebleed (G. L.) in a man twenty-six years old, otherwise in good health and without symptoms, the flow was normal (10.27 grams per 100 cubic centimeters per minute for the right hand, and 10.40 grams for the left) with room temperature 24.2° C. The erythrocyte count was 4,256,000.

[1] Room temperature 23.9° C.

[2] Left hand put into water at 42.5° C.

[3] Left hand put into water at 9° C.

G. L., a man, aged 26 years, height 5 feet, 1½ inches, a laborer, was admitted to the City Hospital on November 6, 1912. He complains of bleeding from the nose and sometimes from the gums. This is the second nosebleed he has had. It began on November 3 and continued for some days, stopping and starting again at irregular intervals. He never suffered from hemorrhage from the nose except on these two occasions. No family history of hemophilia. His gums are anemic and spongy in the anterior portion. Pyorrhea alveolaris is present. Teeth fairly good, though neglected. Physical examination of the chest negative. The edge of the liver is palpable, otherwise examination of the abdomen is negative.

The blood flow in the hands was measured on November 7, 1912. His nostrils were plugged with cotton and the bleeding had almost stopped. He walked into the room. Hands in bath at 2.38 P.M., in calorimeters at 2.47¾ P.M., and out of calorimeters at 3.02. Pulse (sitting) 112.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.47	31.635°	31.52°		2.56	31.86°	31.755°	
2.49	31.63°	31.53°	24.2°	2.57	31.90°	31.81°	24.2°
2.50	31.65°	31.54°	24.15°	2.58	31.95°	31.87°	
2.51	31.66°	31.55°	24.1°	2.59	32.00°	31.94°	24.3°
2.52	31.68°	31.57°		3.00	32.08°	31.98°	
2.53	31.71°	31.60°	24.15°	3.01	32.11°	32.04°	24°
2.54	31.76°	31.655°		3.02	32.17°	32.08°	
2.55	31.80°	31.695°	24.1°	3.16	31.98°	31.895°	

Cooling of the calorimeters in fourteen minutes, right 0.19° C., left 0.185° C. Volume of right hand 362 c.c., of left 360 c.c. Water equivalent of calorimeters with contents, right 3,385, left 3,383. Rectal temperature 38.7° C. Blood pressure, right arm, systolic 127, 84 (sound gone). Another observation, systolic 128, 110 (sound suddenly diminished), 86 (sound gone).

Plesch (2) has stated that the output of the left ventricle per minute (the minute volume), measured by a gasometric method, is increased in the cases of anemia investigated by him, including pernicious anemia, chlorosis, and secondary anemias. His explanation is that the deficiency of hemoglobin entails the circulation of a greater quantity of blood through the lungs in order that the oxygen requirement of the body may be filled. He does not discuss the mechanism by which this necessary increase in the minute volume is obtained. My observations on the hand flow indicate in an interesting manner that an important, if not the sole, way in which the increased minute volume of the heart is rendered possible

is the diminution in the flow through the periphery by vasoconstriction. This obviously will shorten the average circulation time of the blood and permit its quicker return to the right heart and therefore its more rapid circulation through the lungs. It is to be expected that the cutaneous vessels should be particularly affected by this vasoconstriction. For in the main the blood circulates in the skin, not in the interests of the nutrition of the skin itself, for which a comparatively small flow would suffice, but to regulate the loss of heat. Where it is important that the blood should pass in a fuller stream than ordinary through the lungs it can accordingly be readily spared from the peripheral layers of the body. Since the metabolism in the anemic body is usually less than normal, a smaller cutaneous circulation than normal is in any case required to eliminate the heat produced. If the peripheral vasoconstriction is accompanied by a vasodilatation in the deeper and shorter vascular paths, especially in those passing through the viscera in which a free circulation must be steadily maintained if the metabolism is to proceed normally, the increase in the minute volume of the heart may be all the more easily maintained. Although it may be that the doctrine of reciprocal innervation of the periphery and the deeper parts cannot now be maintained in any very thoroughgoing fashion, there is no question that reciprocal relations can and do exist. The relatively low blood pressure in many of these cases, shows, in so far as it does not depend upon cardiac weakness, that the vasomotor block is not a universal block like that of asphyxia. Where the total volume of the blood is increased, as in chlorosis (3), the increased minute volume of the heart necessitated by the deficiency in hemoglobin may be obtained with little if any diminution in the flow through the skin. For example, if the blood volume were doubled and the hemoglobin percentage halved, the total hemoglobin would be unaltered. It might then be possible that without diminution in the flow through the peripheral parts, the flow through the lungs could be doubled and the oxygen requirement completely met. In this case the quantity of hemoglobin passing through the hand, per unit of time, might be reduced to half without change in the blood flow, while the quantity of hemoglobin passing through the lungs would remain normal. In E. D., for example, the hemo-

globin passing through 100 c.c. of hand per minute would correspond only to that contained in three grams of normal blood, a very marked deficiency in the hemoglobin circulation through the hands for a girl of sixteen. In O. L., also, the apparently fair flow of about seven grams of blood per 100 cubic centimeters of hand only corresponds to the hemoglobin of about three grams of normal blood.

In A. P., whose hand blood flow appears to be normal, the hemoglobin going through 100 c.c. of hand per minute is equivalent only to that contained in about six grams of normal blood, a distinct although not a very great deficiency.

We shall not discuss the question whether an increase in the minute volume of the heart in the anemias is not obtained in part or chiefly by an acceleration in its rate. For an increase in minute volume occasioned in this way would equally affect the peripheral circulation unless a redistribution of the blood were brought about by vasomotor action in the manner already suggested. Besides, considerable difference of opinion exists among observers as to the effect of changes in the rate of the heart on its output (4), and as to the changes in the output which are possible. Table I shows clearly that there is no direct relation between the pulse rate and the blood flow through the hand. The highest pulse rate of the series, 120 in H. W., was associated with one of the lowest hand flows, the lowest pulse rate, 52 in J. B., with a fair although subnormal flow. The independence of the hand flow and the pulse rate in different cases is still better displayed in table II, embodying the results on a case of bradycardia, a case diagnosed as paroxysmal tachycardia, a case showing jaundice with a slow pulse, and a case showing jaundice with normal or somewhat accelerated pulse rate.

X., a man, aged 32½ years, height 6 feet, weight 170 pounds. Bradycardia. The first examination of the flow in the hands was made on June 4, 1912. He feels quite comfortable to-day; sometimes he has headaches. He drove his automobile in coming to be examined. He was in the room fifteen or twenty minutes before the observation began. The pulse rate (sitting) was then 37 per minute, on the average of a four minute period. Hands in bath at 1.12½ P.M., in calorimeters at 1.24½, and out at 1.40 P.M. Mouth temperature 36.7° C.

TABLE I.

Case.	Age in yrs.	Date.	Pulse rate.	Blood pressure.	Temperature (C.) of				Hand volume in c.c.		Heat given off in small calories.			Blood flow in gm. per min.		Flow per 100 c.c. of hand per min.		Notes.
					Room.	Arterial blood.	Calorimeters.											
							Right.	Left.	Right.	Left.	Right.	Left.	In min.	Right.	Left.	Right.	Left.	
H.W.	24	Apr. 18, 1911	120		23.5°	37.35°	30.19°	30.20°	412	378	931	753	17	8.49	6.88	2.06	1.82	Pernicious anemia. R. B. C. 2,000,000.
D.K.	44	July 2, 1912	84	103,(82)[4]60	29.1°	37.7°	32.05°	32.03°	480	476	3,670	3,667	18	40.09	39.92	8.35	8.38	Pernicious anemia. R. B. C. 1,648,000. Hemoglobin 45.
							32.54°				191		3	13.70		2.85		Left hand in cold water.
					29.2°		32.67°				957		7	30.20		6.29		Left hand still in cold water.
							32.80°				192		2	21.79		4.54		Left hand in warm water.
							33°				1,478		8	43.67		9.10		Left hand still in warm water.
S.K.	48	Jan. 24, 1913	108		23.3°	37.15°	31.17°	31.14°	330	324	225	208	10	4.18	3.84	1.26	1.18	Carcinoma. R. B. C. 2,112,000. Hemoglobin 45.
F.W.	22	Dec. 27, 1912	100	124,73	24.7°	36.75°	31.55°	31.45°	449	403	1,589	1,145	15	22.63	16.00	5.04	3.94	Hemophilia.
					24.2°		31.80°				1,126		11	22.97		5.11		Left hand in warm water.
					24.2°		31.92°				539		11	11.27		2.79		Left hand in cold water.
M.A.	38	Jan. 28, 1913	80	118,(85)[4]	23.1°	36.85°	31.17°	31.15°	330	332	1,031	1,206	13	15.51	18.08	4.70	5.44	R. B. C. 3,000,000. Hemoglobin 80.
E.D.	16	Feb. 5, 1912	130 / 96	105,(85)[4]73	22.2°	37°	31.21°	31.19°	303	275	1,285	1,077	13	18.96	15.84	6.25	5.76	Chlorosis. R. B. C. 4,100,000. Hemoglobin 50.
					22.5°		31.32°				320		5	12.51		4.12		Left hand in warm water.
					22.4°		31.40°				930		9	20.50		6.76		Left hand still in warm water.
A. P.	32	Feb. 4, 1913	104 / 88	115,63	23.5°	37°	31.12°	31.03°	390	386	1,806	1,678	10	34.12	31.23	8.75	8.09	R. B. C. 4,600,000. Hemoglobin 70.
T.	30	Jan. 11, 1911	72		22.6°	37.6°	30.58°	30.55°	275	245	1,466	1,456	13	17.84	17.65	6.48	7.20	R. B. C. 4,584.000. Hemoglobin 40.

Case.	Age in yrs.	Date.	Pulse rate.	Blood pressure.	Temperature (C.) of				Hand volume in c.c.		Heat given off in small calories.			Blood flow in gm. per min.		Flow per 100 c.c. of hand per min.		Notes.
					Room.	Arterial blood.	Calorimeters.											
							Right.	Left.	Right.	Left.	Right.	Left	In min.	Right.	Left.	Right.	Left.	
O.L.	36	Apr. 27, 1911	96		25.4°	37.3°	29.64°	29.58°	470	448	2,048	1,934	9	33.00	30.93	7.02	6.90	Gastric hemorrhage. R. B. C. 2,250,000. Hemoglobin 45.
					25.4°		29.95°				555		3	27.97		5.95		Left hand in warm water.
					25.4°		30.20°				1,492		5	46.69		9.93		Left hand still in warm water.
					25.4°		30.47°				798		5	25.96		5.52		Left hand in cold water.
					25.3°		30.65°				764		4	31.91		6.79		Left hand still in cold water.
					25.3°		30.86°				1,076		6	30.94		6.58		Left hand dried and wrapped.
J. B.	34	Apr. 4, 1912	52		23.9°	36.7°	30.63°	30.67°	407	410	1,231	1,403	10	22.53	25.85	5.53	6.30	Scurvy.
							30.79°				55		1	10.34		2.54		Left hand in warm water.
					23.9°		30.99°				1,642		7½	42.60		10.47		Left hand still in warm water.
							31.21°				342		3	23.07		5.66		Left hand in cold water.
							31.42°				1,573		8	41.39		10.17		Left hand still in cold water.
							31.61°				205		2	22.37		5.50		Left hand dried and wrapped.
							31.71°				684		3	50.77		12.47		Left hand still wrapped up.
G. L.	26	Nov. 7, 1912	112	128,(110)[4]86	24.2°	38.2°	31.93°	31.83°	362	360	2,098	2,148	10	37.18	37.46	10.27	10.40	Nosebleed. R. B. C. 4,256,000.

[4] The numbers in brackets are the pressures at which the abrupt decline in the sound occurred; the smaller numbers following are the pressures at which the sound disappeared.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
1.24	31.20°	31.15°		1.33	31.68°[5]	31.70°	
1.25	31.20°	31.17°	24.6°	1.34	31.79°	31.75°	24.6°
1.26	31.27°	31.24°		1.35	31.82°	31.80°	
1.27	31.34°	31.31°	24.8°	1.36	31.87°	31.85°	
1.28	31.395°	31.36°		1.37	31.91°	31.91°	
1.29	31.44°	31.42°		1.38	31.96°	31.95°	24.6°
1.30	31.51°	31.48°	24.7°	1.39	32.01°	32.00°	
1.31	31.58°	31.56°		1.40	32.04°	32.03°	
1.32	31.65°	31.63°		2.04	31.76°	31.755°	

Cooling of calorimeters in twenty-four minutes: right 0.28° C., left 0.275° C. Volume of right hand 493 c.c., of left hand 469 c.c. He is right handed. Pulse (sitting) at end of the blood flow examination 34, average of three minutes. Water equivalent of calorimeters with contents, right 3,489, left 3,470.

Second examination on June 11, 1912. He drove over as on the last occasion. But today his pulse was counted two or three minutes after he came. The rate was 51, average of three minutes. For the first two minutes it was 48 per minute and for the third minute 57. He said that for the last minute he got to thinking about something which constituted a problem for him.

Hands in bath at 1.08 P.M., in calorimeters at 1.19 and out of calorimeters at 1.38. 3,015 c.c. of water in each calorimeter. Mouth temperature 37° C.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
1.18	31.125°	31.04°	24°	1.30	31.65°	31.51°	
1.20	31.18°	31.09°		1.31	31.695°	31.56°	24.6°
1.21	31.24°	31.15°		1.32	31.775°	31.63°	
1.22	31.30°	31.21°	24.6°	1.33	31.86°	31.72°	24.7°
1.23	31.33°	31.235°		1.34	31.90°	31.75°	
1.24	31.39°	31.29°	24.6°	1.35	31.95°	31.79°	24.7°
1.25	31.42°	31.32°		1.36	31.98°	31.82°	
1.26	31.46°	31.35°		1.37	32.01°	31.84°	24.7°
1.27	31.525°	31.405°	24.5°	1.38	32.02°	31.85°	
1.28	31.58°	31.45°		1.55	31.81°	31.65°	
1.29	31.61°	31.47°	24.5°				

Cooling of calorimeters in seventeen minutes, right 0.21°, left 0.20°. Volume of right hand 477 c.c., of left 444 c.c. Pulse (sitting) at end of examination, 34 and 35 in two successive minutes. Water equivalent of calorimeters with contents, right 3,476, left 3,450.

In the first blood flow examination with a pulse rate of 35 the flow in X. was 10.37 grams per 100 cubic centimeters per minute in

[5] The stirring in the right calorimeter was known to be deficient here.

TEXT-FIG. 1. Part of a polygraph tracing from X., taken May 30, 1912. The lower trace is the radial, the upper the jugular.

the right hand, and 10.93 grams in the left hand, with a room temperature of 24.7° C. A week later, with a somewhat higher average pulse rate and a room temperature of 24.5° C., the flows were respectively 8.75 grams, and 8.32 grams. On the first day he had in accordance with his usual custom for some time taken no lunch, and had rested for a while after coming to the hospital. On the second day he had had lunch and was examined immediately on his arrival. The case was one of true bradycardia, not of heart block, the jugular tracing showing a number of *a* waves equal to the number of beats in the radial artery (text-figure 1).[6]

The patient had been a heavy smoker.

Dr. Carter writes: "He has been under my care since 1907. It was in the spring of 1910 that he had his first syncopal attacks associated with a pulse, at that time of 37 to 42. In the fall of 1910 I sent him to Dr. Thayer in Baltimore for observation." Dr. W. S. Thayer (6) has described this case as one of "permanent bradycardia with no apparent cardiac abnormality. After atropin ($\frac{1}{30}$ of a grain subcutaneously) the pulse rose in twenty minutes to 60. Numerous sphygmographic tracings showed normal auricular and ventricular contractions with no delay in the *a-c* time." April 18, 1913. His pulse rate today is 49 and 46 in two successive minutes. He had just been "running around," he said, so that probably there is no change in rate. The acceleration at the end of inspiration is well marked, as was noted by Dr. Thayer, showing that inhibitory tone is present. A month before he had been operated on for appendicitis under nitrous oxide anesthesia and had made a normal recovery.

In table II is next shown a case (F. R.), a woman aged 52, with a pulse rate of 156 and a blood flow of only 4.78 grams per 100 cubic centimeters per minute for the right hand, and 4.30 grams for the left, although the room temperature was 26° C. This pulse rate indicates complete or almost complete inactivity of the inhibitory mechanism. It is of interest as a sign of concomitant inactivity, as regards reflex changes, of the vasomotor centers that the flow in one hand is practically unaffected by the application of heat or cold to the contralateral hand. So slight a reflex effect as that witnessed in this patient is seldom seen.

Mrs. F. R., aged 52 years, height 5 feet, dispensary patient. Diagnosis "paroxysmal tachycardia." She was never ill until two years ago, when "heart trouble" began. Slight pain in left inframammary region. No enlargement of the heart. No murmurs. Lungs clear. Pharynx reddened. Reflexes normal.

[6] I am indebted to Dr. E. S. Carter for the tracing, a portion of which is reproduced in the figure, and for the opportunity of studying the case.

TABLE II.

Case.	Age in yrs.	Date.	Pulse rate.	Blood pressure.	Temperature of				Hand volume in c.c.		Heat given off in small calories.			Blood flow in gm. per mim.		Flow per 100 c.c. of hand per min.		Notes.
					Room.	Arterial blood.	Calorimeters.											
							Right.	Left.	Right.	Left.	Right.	Left.	In min.	Right.	Left.	Right.	Left.	
X.	32½	June 4, 1912	35		24.7°	36.7°	31.62°	31.60°	493	469	3,506	3,531	15	51.12	51.28	10.37	10.93	After rest. No lunch.
		June 11, 1912	51, 35		24.5°	37°	31.60°	31.47°	477	444	3,650	3,312	18	41.72	36.97	8.75	8.32	After lunch, without rest.
F. R.	52	May 25, 1911	156		26°	38.1°	30.12°	30.03°	326	335	1,007	942	9	15.58	14.41	4.78	4.30	
							30.35°				940		8	16.84		5.16		Left hand in warm water.
							30.58°				1,040		10	15.36		4.71		Left hand in cold water.
G.V.	44	Sept. 27, 1912	63	105,69	22.1°	37.1°	31.04°	30.98°	473	467	1,320	1,284	19	12.73	12.27	2.69	2.62	
S.L.	35	Jan. 8, 1913	84	117,80 (100)[7]	21.7°	36.75°	31.77°	31.69°	494	467	4,938	4,718	15	73.45	69.06	14.86	14.78	
					22.4°		32.40°				350		2	44.70		9.05		Left hand in cold water.
					22.2°		32.66°				2,058		8	69.88		14.14		Left hand still in cold water.
					22.1°		33.16°				2,495		10	77.22		15.63		Left hand in warm water.

[7] The numbers in brackets are the pressures at which the abrupt decline in the sound occurred; the smaller numbers following are the pressures at which the sound disappeared.

She has had thirteen children, nine of whom are alive. She nursed them all. The menopause occurred two years ago. She is corpulent, has shortness of breath on exertion, and attacks of palpitation, especially when excited. Rapid pulse, 165 a minute. No arrhythmia. Three times in the last two years she became unconscious, the first time for two hours. During these attacks her feet, according to her daughter, were quite cold, and were put into hot water. Her hands now feel rather cool, indeed considering the external temperature, quite cool. She has had hard luck and much worry. No thyroid enlargement, no exophthalmos. The symptoms and signs (rapid pulse, increased temperature, and peripheral vasoconstriction) suggest early Graves' disease.

Blood flow in the hands examined May 25, 1911. Mouth temperature 38.1° C. Hands in bath at 2.23 P.M., in calorimeters at 2.33½ P.M. At 2.44 P.M. left hand put into water at 43° C., at 2.52 into water at 9° C.

Time.	Temperature of Calorimeters. Right.	Temperature of Calorimeters. Left.	Room.	Time.	Temperature of right calorimeter.	Time.	Temperature of right calorimeter.	Notes.
2.33	29.95°	29.895°		2.46	30.285°	2.57	30.595°	
2.35	30.005°	29.91°		2.47	30.305°	2.58	30.62°	
2.36	30.35°	29.925°		2.48	30.345°	2.59	30.635°	
2.37	30.06°	29.95°	25.9°	2.49	30.38°	3.00	30.66°	
2.39	30.10°	30.005°		2.50	30.40°	3.01	30.68°	Room temperature 26.1°.
2.40	30.13°	30.025°		2.51	30.43°			
2.41	30.16°	30.04°		2.52[8]	30.46°	3.02	30.695°	Hand out of calorimeter at 3.02.
2.42	30.19°	30.095°		2.53	30.48°			
2.43	30.21°	30.11°	26.1°	2.54	30.495°	3.03		Left 30.03°.
2.44	30.24°	30.135°		2.55	30.53°	3.15	30.60°	Left 29.94°.
2.45	30.27°			2.56	30.57°			

Cooling of calorimeters, right 0.095° in 13 min., left 0.195 in 31 min., 0.09° in 12 min.
Volume of right hand 326 c.c., of left 335 c.c.
Hands are fat. Pulse 156 (sitting).

The third case in table II, G. V., a man 44 years old, with long standing jaundice and a pulse rate of 63, had a flow in the right hand of only 2.69 grams per 100 cubic centimeters per minute, and in the left 2.62 grams, with room temperature 22.1° C. Anemia was probably a factor in the small peripheral flow. His hands were always cold to the touch. Whether the itching of the skin of which he complained was concerned in the vasoconstriction is unknown.

G. V., a man, aged 44 years, a Hungarian laborer, was admitted to the City Hospital on September 24, 1912. He came in for treatment of a left inguinal hernia which he has had for four years. He also sought the hospital on account

[8] Although the cold water was purposely made one degree higher than usual, she feels it very uncomfortable and complains of it. Her hand had to be taken out of the cold water intermittently for the first 2 or 3 minutes.

of weakness. He is married but has no children. His wife has had no miscarriages. He has had general jaundice for the past eight years. Examination of thorax negative. Liver enlarged, left lobe palpable three fingers' breadth above the umbilicus. Spleen enlarged to percussion, palpable and tender. Skin itches considerably.

Blood flow in hands measured September 27, 1912. Hands in bath at 2.49 P.M., in calorimeters at 2.59, and out of calorimeters at 3.25.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.57½	31.03°	30.98°		3.14	31.00°	30.95°	22.1°
3.00	30.99°	30.94°		3.15	31.01°	30.96°	
3.01	30.98°	30.93°		3.16	31.015°	30.96°	
3.02	30.97°	30.925°		3.17	31.02°	30.965°	22°
3.03	30.97°	30.92°		3.18	31.025°	30.975°	
3.04	30.96°	30.92°	22.1°	3.19	31.04°	30.98°	
3.06	30.96°	30.91°		3.20	31.06°	30.995°	22.2°
3.07	30.96°	30.91°		3.21	31.08°	31.01°	
3.08	30.96°	30.91°	22.3°	3.22	31.09°	31.02°	
3.09	30.965°	30.905°		3.23	31.095°	31.04°	22.2°
3.10	30.97°	30.91°		3.24	31.10°	31.05°	
3.11	30.98°	30.92°	22.2°	3.25	31.10°	31.045°	
3.12	30.99°	30.93°		3.54	30.73°	30.685°	
3.13	30.995°	30.945°					

Cooling of calorimeters in twenty-nine minutes, right 0.37° C., left 0.36° C. Pulse (sitting) 63. Volume of right hand 473 c.c., of left hand 467 c.c. Rectal temperature 37.6° C. Blood pressure, left arm, systolic 104 (palpation), 105 (stethoscope), 69 (sound gone). Another observation: 105, 69. Water equivalent of calorimeters with contents, right 3,473, left 3,469.

In S. L., a case of jaundice with a normal pulse rate, or perhaps a rate somewhat accelerated, the flow in the hands was even greater than normal for the room temperature of 21.7° C.,—14.86 grams per 100 cubic centimeters per minute for the right hand, and 14.78 grams for the left. An increase in the hand flow in disease is much less common than a decrease. The vasomotor reflexes were marked. The increased vasodilatation in the right hand (whose blood vessels must have been already considerably dilated) on immersing the left hand in warm water is especially notable. The flow increased to 15.63 grams per 100 cubic centimeters per minute. Immersion of the left hand in cold water caused a transient diminution of the flow in the right (to 9.05 grams for the first two minutes) followed by an increase to 14.14 grams per 100 cubic centimeters per minute for the remaining eight minutes.

S. L., aged 35, a Slovak, was admitted to the City Hospital on January 1, 1913. He has been sick for two months with pain in the stomach and weakness, but he has not vomited. Never any blood in the stools. He has lost weight to a considerable extent in the past two months, during which he has worked at the National Carbon Works. He has had a cough and several nosebleeds. For the past six or seven months he has been getting more and more jaundiced, but since he entered his present employment the jaundice has been increasing rapidly. He used to drink whiskey moderately. Physical examination of chest and heart shows nothing special. Pulse regular, good tension, good volume, some sclerosis. The abdomen is very rigid and distended. The liver is enlarged, extending to the umbilicus, hard to the touch, not tender. No nodules present. The spleen is also enlarged to the umbilicus and hard but not tender or nodular. A space can be defined between the liver and spleen. Examination of the kidneys negative. Some fluid present in the abdomen. Blood examination: leucocytes 8,000 (polymorphonuclears 69.6, eosinophils 2.6, large mononuclears 3.1, small mononuclears 21.8, transitionals 0.8). The color of the skin, etc., is yellow, not greenish. Diagnosis: hypertrophic biliary cirrhosis.

Blood flow in the hands examined on January 8, 1913. Hands in bath at 2.38 P.M., in calorimeters at 2.49, and out of calorimeters at 3.25. Pulse (sitting) 84. At 3.06 P.M. left hand put into water at 8° C. He feels it very cold. At 3.15 P.M. left hand put into water at 43° C.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.47½	31.13°	31.07°					
2.50	31.17°	31.12°		2.58	31.82°	31.74°	21.8°
2.51	31.24°	31.19°		2.59	31.895°	31.81°	
2.52	31.325°	31.27°		3.00	31.97°	31.88°	22°
2.53	31.415°	31.36°	21.2°	3.01	32.06°	31.96°	
2.54	31.50°	31.43°	21.3°	3.02	32.14°	32.05°	21.8°
2.55	31.58°	31.50°	21.4°	3.03	32.22°	32.13°	
2.56	31.66°	31.58°	21.5°	3.04	32.28°	32.19°	
2.57	31.73°	31.66°		3.05	32.36°	32.26°	
3.06	32.39°		22.4°	3.16	32.935°		22.1°
3.07	32.43°			3.17	32.98°		
3.08	32.495°		22.4°	3.18	33.03°		22.2°
3.09	32.56°			3.19	33.09°		
3.10	32.61°		22.2°	3.20	33.14°		22.2°
3.11	32.67°			3.21	33.20°		
3.12	32.72°		22.15°	3.22	33.26°		22°
3.13	32.79°			3.23	33.32°		
3.14	32.84°		22.2°	3.24	33.38°		22.2°
3.15	32.89°			3.25	33.435°		
				3.40	33.18°	31.75°	

Cooling of calorimeters, right 0.255° in fifteen minutes, left 0.51° in thirty-five minutes. Volume of right hand 494 c.c., of left 467 c.c. Blood pressure, left arm, systolic 117 (palpation), 117 (stethoscope), 80 (sound gone). The sound became indistinct a little below 100, but did not actually disappear till about 80.

The veins of the arms are large. Both hands feel warm to the touch and equally so. The whole skin is warm. Rectal temperature 37.27° C. Water equivalent of calorimeters with contents, right 3,490, left 3,469.

In conclusion it should be pointed out, perhaps, that if in anemia peripheral vasoconstriction, especially in the cutaneous vessels, is a compensatory mechanism for increasing the stream of blood in the lungs, disorders of the skin caused or perpetuated by deficient circulation are rationally treated by measures which augment the hemoglobin content of the blood. When the deficiency of hemoglobin is remedied the deficiency in the cutaneous blood flow will take care of itself. It has long been known and is a commonplace of practice that many skin conditions are benefited by general treatment of the coëxisting anemia where local treatment alone is inefficacious. Local treatment of limited portions of the skin, however, aimed at increasing the blood flow in that region would not be contraindicated from the point of view of the compensatory action of a general peripheral vasoconstriction. For vasodilatation in a limited area of the skin, while it might greatly benefit the local condition, would not appreciably affect the pulmonary flow.

Where anemia is excluded it is probable that the hand flow under standard conditions which eliminate as far as possible accidental local vasoconstriction is an index to the minute volume of the heart, or, rather, gives the means in successive observations of following to some extent changes in the minute volume. This seems to follow from the fact that in one and the same healthy individual and in pathological cases where the clinical state has undergone no sensible change, the hand flow varies only within narrow limits when the subject is under a steady regimen, and the controllable external conditions are the same. The fact then that X., who had no anemia, with a pulse rate of 35, had a hand flow entirely within the normal range for his age may be taken to indicate that his minute volume under the given external conditions was normal. The output per beat must, therefore, have been twice that of a normal man of the same weight. Nothing, of course, can be said from these observations as to the power of his heart to respond to such increased requirements as occur during considerable muscular exertion. Buchanan (7) has remarked that among athletes very slow pulse rates are not incompatible with great exertion.

CONCLUSIONS.

1. In the cases of anemia studied (pernicious anemia, chlorosis, and secondary anemia) the blood flow in the hand is smaller than the normal flow.

2. Accepting Plesch's result, that the minute volume of the heart is increased in the anemias, the diminution in the hand flow is interpreted as a sign of a vasoconstriction in peripheral parts, which facilitates the increased flow through the lungs.

3. The deficiency in the hand flow is less in the chlorotic group than in the other cases of anemia. The explanation suggested is that the increased total volume of the blood in chlorosis permits the normal amount of hemoglobin to circulate through the lungs without a marked increase in the peripheral vasoconstriction.

4. The view that the diminution in the cutaneous blood flow in anemia is a necessary compensatory arrangement for increasing the flow through the lungs affords a natural explanation of the beneficial effect in many cutaneous disorders of general measures, especially those aimed at improving the quality of the blood.

5. In a case of bradycardia with a pulse rate permanently below 40 during rest in a sitting position, the hand flow was normal.

I am indebted to the Staff of Lakeside Hospital and Dispensary, and especially to the Staff of the City Hospital for many courtesies extended to me during this investigation.

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INTESTINAL OBSTRUCTION.*

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Whipple, Stone, and Bernheim[1] studied the cause of death in dogs with high intestinal obstruction, by producing a closed loop of the duodenum, beginning just below the pancreatic ducts and ending beyond the suspensory ligament. This portion was isolated from the remainder of the intestinal tract by tying a heavy ligature tightly at these two points, and burying it under an inverted layer of the peritoneum. A gastro-enterostomy was then done. Dogs so treated died in from thirty-six to seventy-two hours. The authors inferred from this that there developed in the closed loop a poisonous material, the absorption of which caused death. Since the closed loop contained neither gastric secretion, bile, nor pancreatic juice, the conclusion seemed inevitable that some alteration in the loop function was responsible for death.

A series of careful and well controlled experiments was then undertaken to study the nature, and if possible, to determine the source of the poison. The method employed was to produce the isolated loop, collect the contents, sterilize them by heating to 60° C., filtering them, and then, after autolysis, to inject them into a healthy dog, intravenously, subcutaneously, or intraperitoneally. It was demonstrated in this way that dogs so injected died promptly. Further, the authors were unable to isolate such a poisonous material from the normal intestine by any method of treatment; and if the mucosa of the loop were first destroyed by a fluoride, the poisons did not appear. Lastly, some dogs died within a few days, when the loop was drained externally, there being an absorption directly from the mucosa, and not from the lumen.

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[1] Whipple, G. H., Stone, H. B., and Bernheim, B. M., *Jour. Exper. Med.*, 1913, xvii, 286, 307.

The authors summarize, in part, as follows: "There seems, therefore, no escape from the conclusion that a poisonous substance is formed in this closed duodenal loop which is absorbed from it and causes intoxication and death. Injection of this toxic substance into a normal dog gives intoxication and a reaction more intense than, but similar to that developing in a closed-loop dog."[2] . . . "The mucosa of closed or drained duodenal loops contains a toxic substance quite similar to the toxic material found in the lumen of the closed loops." . . . "Normal intestinal mucosa contains no toxic substance nor can it neutralize *in vitro* the toxic substance produced in the closed loops.[3] . . . Our experiments show that formation and absorption of a toxic substance may proceed in a loop whose blood supply has been in no way disturbed, and whose mucosa is quite intact both in gross and microscopically." The authors apply their findings to the condition of simple intestinal obstruction, and state it as their belief "that the toxic material concerned is the same in both instances."[4] They add: "Our experiments indicate that intoxication is the primary effect, and the loss of body fluids a secondary one, rather than the reverse, as is claimed by Hartwell and Hoguet."[5] In substance, they claim to have proved that a closed loop of intestine, without undergoing any morphological change, produces a toxic substance which is absorbed and causes death. Moreover, they infer that a similar condition exists in simple intestinal obstruction, and that here also death results from the absorption of this same poison.

We fail to find any evidence in their experiments, as published, to justify these claims. On the contrary, we find that the protocols definitely show a damage to the intestinal wall, and that the toxic substance they are dealing with arises from this source. In the absence of this damage no toxemia is present except that produced by the loss of water in the vomitus. We submit the following review of their protocols in support of this view.

Dog S–34.[6]—Moribund in twenty-seven hours. Killed with ether. Autopsy immediately. "The duodenal loop is of enormous size." . . . "Purplish patches show through the transparent serosa. . . ." The contents are blood-stained.

Microscopical Examination.[7]—"In places the wall of the duodenum shows necrosis, hemorrhage, and invasion by leucocytes."

Dog S–27.[8]—Died in thirty-two hours.

Autopsy.—"The duodenal loop is contracted and contains a little material. Its walls show a few areas of subserous hemorrhage, about 1 or 2 cm. in diam-

[2] *Ibid.*, p. 306.
[3] *Ibid.*, p. 323.
[4] *Ibid.*, p. 321.
[5] Hartwell, J. A., and Hoguet, J. P., *Jour. Am. Med. Assn.*, 1912, lix, 82.
[6] Whipple, Stone, and Bernheim, *loc. cit.*, p. 288.
[7] *Ibid.*, p. 289.
[8] *Ibid.*, p. 289.

eter. There is obvious necrosis associated with these areas, and through one of them rupture had taken place, with escape of duodenal loop fluid into the peritoneum." No microscopical examination is given.

Dog S–20.[9]—Death in forty-eight hours. 100 c.c. of fluid are in the loop. "The closed loop mucosa is pinkish and a little swollen, but no ulcers are present." The proximal loop was not tight, and some fluid had probably regurgitated into the stomach.

Dog 56.[10]—Death in fifty-five hours. Remained well for twenty-four hours.

Autopsy.—"The abdominal cavity is clear except for slight granular peritonitis about the loop and area of operation." . . . "The small intestine below the loop contains blood-stained mucus, and its mucosa has a mottled appearance, with large areas of deep red color where the capillary injection is quite marked. The duodenal loop is rather flabby, and not greatly distended. It contains about 50 c.c. of a thin soup-like, faintly blood-tinged fluid. The loop mucosa is quite intact, but of a delicate pinkish tinge." No microscopical examination is given.

Dog S–53.[11]—Death in three days. Seemed well for twenty-four hours.

Autopsy.—The condition of the loop mucosa is not given; it is merely stated that it is pink and intact. No microscopical examination is given.

Commenting on these four experiments the authors say: "The duodenal loop may show a normal intact mucosa in gross and microscopically, except for a little diffuse injection. Again it may show necrosis, ulceration, and hemorrhage, and the contained fluid may vary correspondingly in characteristics, but it always contains a toxic substance."[12] In only one case, however, is a microscopical examination given, and then marked changes were found, while in the others evidence of change was seen grossly. The length of life seems directly to correspond to the lack of damage found in the mucosa, as demonstrated by the reported findings in dog S–53, which lived for three days.

The following protocols give the conditions found in the duodenal loop, when the dog is killed at the end of twenty-four to twenty-eight hours. In one case, dog S–4,[13] the contents were blood-tinged, due to inversion of the duodenal mucosa. "The mucosa throughout the loop is intact, but slightly swollen and of a pinkish color."

Microscopical Examination.—"The tips of a few of the villi are slightly swollen, and contain a few polymorphonuclear leucocytes. The epithelium is normal throughout."

Dog S–2.[14]—In twenty-six hours the duodenal loop contained blood-tinged fluid; in places, on microscopical examination, "it shows some areas of necrosis with hemorrhage and invasion by pus cells, involving mucosa and submucosa."

Dog S–5.[15]—The loop contained blood-tinged fluid.

[9] *Ibid.*, p. 289.
[10] *Ibid.*, p. 289.
[11] *Ibid.*, p. 290.
[12] *Ibid.*, p. 291.
[13] *Ibid.*, p. 292.
[14] *Ibid.*, p. 293.
[15] *Ibid.*, p. 294.

Microscopical Examination.—"The duodenal loop is quite normal." In two dogs, S–28 and S–29,[16] the duodenal loop contents were not blood-tinged, and the mucosa, on gross examination, seemed normal, though no microscopical examination is given.

These dogs were all in fair condition when killed, being described as "dull and drowsy," "weak," "pulse strong," "somewhat dull, but not very toxic." The incipient damage to the mucosa, as shown in these cases by the blood-tinged fluid and the microscopical examination, explains the cause of their condition. We cannot reconcile a normal mucosa with a blood-tinged content, as reported in dog S–5.

Dogs S–12 and S–9.[17]—Both dogs were killed after forty-four hours, and were apparently in fair condition, dog S–9 showing a carotid pressure of 100 to 110 mm. of mercury, with almost normal pulsation. There were no visible changes in the loop mucosa, either on gross examination or microscopically.

Dog S–38.[18]—The dog was killed after forty-eight hours, his condition being "somewhat toxic." "The blood pressure was practically normal and the pulse regular." . . . "The duodenal loop contains 20 to 30 c.c. of a strawberry colored thick fluid. The loop shows a diffuse pinkish mucosa, no ecchymoses, and no ulcers."

These three dogs were killed after from forty-four to forty-eight hours, when in good condition, and no change was found in the mucosa. If the enclosed secretion from the mucosa itself is the active poison, the absorption of which, by the mucosa, causes death, why are the dogs not made more ill? We believe it is because in these individual cases the mucosa was not so severely damaged. If we compare them with group 1, in which the dogs were allowed to die, and with the group immediately preceding, where at the end of approximately twenty-four hours the dogs were apparently more toxic, and a definite change in the mucosa was found, we cannot escape the conclusion, from a careful analysis of the cases, that the severity of the toxic symptoms is in direct ratio to the damage done to the mucosa.

In their injection experiments[19] the authors prove beyond question that they have obtained from the contents of the closed loop a substance, which, after sterilization by heat (60° C. for thirty minutes) and filtering through a Gooch or other porcelain filter, is very poisonous, and will cause death in a few hours, if it be injected

[16] *Ibid.*, p. 293.
[17] *Ibid.*, p. 294.
[18] *Ibid.*, p. 295.
[19] *Ibid.*, pp. 301–304.

into the vein, the peritoneum, or the subcutis of a dog. Autolysis under toluol and chloroform does not destroy the toxic substance. Poisoning by these injections is not evidence that the materials are absorbed from the obstructed intestine, as is pointed out by Hartwell and Hoguet.[20]

Whipple, Stone, and Bernheim find that a similar poison can be extracted from the mucosa (not the loop content) of a non-drained or a drained closed loop, injection of which into dogs produces severe toxic symptoms.[21] They argue from this that the substance with which they are dealing is elaborated in the mucosa of the closed loop, and that some unknown force is at work which profoundly alters the activity of the epithelial cells. Yet they emphasize the fact that there is no demonstrable change from the normal. Where do they conceive this toxic substance to arise? They disprove Draper Maury's contention that it is a normal output of the mucosa, which is poisonous unless neutralized with fluids from other portions of the alimentary tract, by showing that duodenal loop fluid, digested with normal intestinal mucosa, loses none of its toxicity.[22] With a normally secreting mucous membrane, undergoing no change from the normal, it is difficult to conceive how it rapidly forms a very toxic material which is directly absorbed from it into the general circulation, with resulting death. It seems inevitable that severe changes take place in the mucosa,—they are usually demonstrable,—and that the toxic material comes from this alteration in the mucosa. In all cases where death occurs from the closed loop itself, some evidence of damage to the mucosa is described, and the severity of the symptoms runs parallel to the severity of the demonstrable damage.

A careful study, therefore, of the findings of these authors makes us unwilling to accept their conclusion that a poisonous substance is poured out by a normal mucosa, or elaborated and directly absorbed by the normal cells, which substance produces the toxic symptoms in simple high intestinal obstruction. In discussing the results obtained by Hartwell and Hoguet,[23] who kept dogs with a

[20] Hartwell, J. A., and Hoguet, J. P., *Am. Jour. Med. Sc.*, 1912, cxliii, 357.
[21] Whipple, Stone, and Bernheim, *loc. cit.*, pp. 310–311.
[22] *Ibid.*, p. 316.
[23] Hartwell and Hoguet, *Jour. Am. Med. Assn., loc. cit.*

simple obstruction alive by simply replacing, with normal saline solution, the fluid lost in the vomitus, Whipple, Stone, and Bernheim say that the benefit derived "is due to dilution and elimination of the toxic material by this means, and the development of a gradually increasing immunity which can be shown to appear in dogs after intravenous injection of the toxic substance."[24] Yet they report conclusive experiments showing that their toxic substance exists neither in the blood of a closed-loop dog, nor remains for two hours in the blood of a dog that has received a lethal dose intravenously.[25] It is difficult to comprehend, then, how saline solution given once a day is capable of washing out a substance from the blood, which, within two or three hours, is "fixed by the cells or partly destroyed," and which, if not washed out, will inevitably cause death. If this were possible, one ought to find a cure for organic poisons by a hypodermoclysis, and, similarly, the closed-loop dogs ought to be saved by this means.

In commenting on the claim of Hartwell and Hoguet,[26] that this action of salt solution is a strong argument against an essential toxemia, and that the loss of water is the important factor, Whipple and his associates say: "One might argue in the same manner that the appearance of sugar in the urine is the cause of diabetes." The analogy is incorrect. Were it possible to alleviate diabetes by the subcutaneous injections of sugar, to the extent that obstruction may be alleviated by injections of saline solution, the analogy would be acceptable, the loss of sugar being the prime factor in one case, and the loss of water in the other.

Moreover, the most potent argument of Hartwell and Hoguet[27] in this connection,—a normal starving metabolism when saline is given, and a completely deranged one when it is withheld, as determined by Benedict,—is entirely ignored by Whipple, Stone, and Bernheim.

It is our contention that the experiments cited by these authors have no bearing on the subject of uncomplicated obstruction. They show, however, that a damaged intestine will produce a substance which is very poisonous if it gain access to the blood.

[24] Whipple, Stone, and Bernheim, *loc. cit.*, p. 321.
[25] *Ibid.*, pp. 309–310.
[26] Hartwell and Hoguet, *Jour. Am. Med. Assn., loc. cit.*
[27] *Ibid.*

We feel, therefore, that instead of reversing the findings of Hartwell and Hoguet,[28] the experiments of Whipple, Stone, and Bernheim give additional proof to the belief of the former that there is no primary toxemia in intestinal obstruction in the absence of a complicating injury to the intestinal wall.

Since clinical intestinal obstruction is almost invariably associated with a damaged intestine, it is fair to assume that a poison is produced with this condition. That this poison can cause a great flow of fluid into the intestinal lumen with vomiting, is also accepted, and is an important fact. We have no evidence, however, that this is the only factor at work in producing this outflow, and from the fact that the latter takes place in simple obstruction, when no toxic substance is present, almost entirely from above the obstructed point, it is probably due to the irritation on the mucosal cells of the retained secretions of the glands of the stomach, liver, pancreas, and duodenum. The closed-loop dogs vomit a little, but simple obstruction dogs often vomit 10 per cent. of their body weight in one day.

We therefore consider that the lead of Hartwell and Hoguet must be followed in differentiating obstruction alone from obstruction with damage to the intestinal wall, however slight, if accurate results are to be obtained. As yet no one has reported the production of a closed loop in the upper intestine without considerable damage to the wall, and the experiments are therefore not applicable to simple obstruction.

The following results of a low obstruction bear out our contention that the poison arises from a damaged intestine. In these cases saline solution injected subcutaneously will not save life.

Dog 85.—The obstructing clamp was applied to the ileum 63 cm. above the cecum. The dog received large amounts of saline, but died in forty-four hours.

Autopsy.—The intestine is enormously distended for 160 cm. above the obstruction. This part of the intestinal wall is markedly damaged, there being numerous hemorrhages in the mucosa extending outward to the peritoneum.

Microscopical Examination.—The mucous membrane is destroyed. Edema is present throughout all coats. A beginning purulent exudate is seen in the intestinal wall.

Dog 86.—The clamp is applied 40 cm. above the cecum. The dog remained in excellent condition for three days, having vomited only 300 c.c. and having received 1,100 c.c. of saline subcutaneously. After this he grew rapidly sick, and was killed when nearly moribund two days later.

[28] *Ibid.*

Autopsy.—No peritonitis. Obstruction complete. The intestine is moderately distended for 50 cm. above the clamp. There is no evidence of gross damage to the intestinal wall, except immediately above the clamp, where there is a hemorrhagic area in the mucosa 2 cm. in diameter, with beginning necrosis.

Microscopical Examination.—There is an almost complete necrotic destruction of mucosa through this area. The other layers are edematous and show acute exudative inflammation.

Dog 87.—Obstruction of the lower ileum. The dog remained in excellent condition for seventeen days, when he was killed with ether. During this time his total output was 3,950 c.c., and he received 6,600 c.c. of saline, subcutaneously, an average respectively of 232 and 388 c.c. per day, which is less than half the quantities found in a high obstruction.

Autopsy.—Obstruction complete. No peritonitis. At only one point, about 20 cm. above the obstruction, was there a gross change in the intestine. Here a superficial ecchymosis, 5 mm. in diameter, was found. The intestine above the clamp was only moderately distended.

Microscopical Examination.—The spleen, kidneys and liver are normal except for slight congestion. The intestine shows a slight congestion, but otherwise is normal.

A study of these cases clearly demonstrates that the length of life is in inverse ratio to the damage occurring in the intestine. All the animals were under practically identical conditions as far as the stagnation of the intestinal contents is concerned, and if the poison arose from this source, or from functional changes in the mucosa, they should have lived approximately the same time. A simple explanation is forthcoming for the varying damage to the intestine. When antiperistalsis is sufficient to empty the intestine toward the stomach, no damage results. When this is absent the accumulated secretions distend the bowel until the circulation is obstructed and the damage results. The administration of saline subcutaneously exerts no influence either on the production of the poison under this condition, its absorption, or its elimination, and the dogs sicken and die in spite of this treatment. The following case of duodenal obstruction demonstrates this further.

Dog 103.—The middle of the duodenum was sectioned and the ends were closed by inversion. 500 c.c. of saline were given daily, and the animal remained in perfect condition for ten days. In addition it was given all the water it would drink, which was taken in large quantities. On the eleventh day the dog suddenly sickened and was killed with ether when nearly moribund.

Autopsy.—Performed immediately. Obstruction complete. No peritonitis. The stomach and duodenum are greatly distended. The pyloric region shows very severe damage to the mucosa, there being large patches of hemorrhagic staining, with beginning ulceration. A similar condition exists in the duodenum.

Microscopical Examination.—The kidney shows marked congestion, and a severe grade of granular degeneration in the cells. Similar changes are found in the liver. In the intestine below the obstruction there is only slight congestion. Above the obstruction the walls are much thickened. All the coats show edema. There is marked exfoliation of the epithelial cells of the mucosa. A purulent infiltration of the mucosa and submucosa is present. In this case the added distention of the stomach and duodenum resulting from the free ingestion of water caused a severe circulatory disturbance with consequent damage to the mucosa. The subcutaneous injection of saline had no effect on the absorption or the elimination of the resulting poison, and the dog died.

Dogs similarly treated, but not receiving water by mouth, do not sicken, and will live indefinitely except for starvation. Autopsy then shows a normal intestine and gastric mucosa.

Bunting and Jones,[29] in describing the results of their experiments on intestinal obstruction in rabbits state that the animals lived only a few days, and that they did not vomit or lose any considerable amount of water. They argue, therefore, that the theory which considers the loss of water as the essential cause of death cannot be true for rabbits. We cannot accept this evidence as bearing on the condition in dogs, though Bunting and Jones seem to do so. Many rabbits will die in six days from simple starvation, even if given water freely (Mendel and Rose).[30] Bunting and Jones starved the rabbits for one to two days before operation. A major operation was done, and the animals then received neither water nor food. Death might be expected, therefore, in a few days, in many of the rabbits the only explanation being inanition. An animal that has such a precarious hold on life is unsuited for this work, and the results cannot be compared with those found in dogs, as the latter readily withstand starvation for periods of many weeks.

Hartwell and Hoguet[31] reported a dog as in no way inconvenienced by a complete pyloric obstruction for a period of fifteen days. Bunting and Jones found that rabbits so treated died in one day. Obviously there is a difference in the animals, and the results obtained with rabbits can not be used to contradict those obtained in dogs.

Our experiments show many other examples which might be cited to show that in the absence of a damage to the intestine no

[29] Bunting, C. H., and Jones, A. P., *Jour. Exper. Med.*, 1913, xvii, 192.
[30] Mendel, L. B., and Rose, W. C., *Jour. Biol. Chem.*, 1911–12, x, 213.
[31] Hartwell and Hoguet, *Am. Jour. Med. Sc., loc. cit.*

symptoms of poisoning arise, provided the water lost by vomiting is replaced, and in the presence of such damage no amount of saline will, to an appreciable extent, affect the development of poisoning, or prevent death. The conclusion seems inevitable that the only poison present in intestinal obstruction arises from the damage secondary to the obstruction, and not from the stagnation of intestinal contents or an altered function of a normally appearing mucosa.

As final proof of our position, we may report that by exercising great care in avoiding damage to the intestinal wall, we find it possible to keep dogs with the closed loop alive for comparatively long periods. A further communication will deal with these experiments in detail.

The application of these facts to intestinal obstruction in man must be made with reserve. The human intestine is apparently incapable of withstanding the same amount of distention without damage, and consequently a poisoned condition occurs earlier in man. However, there are two important results of this experimental work. The need of large amounts of saline subcutaneously is proved, and has been used by us with advantage, patients having readily absorbed three to six quarts in twenty-four hours. There is no necessity of draining out the intestinal contents unless the bowel is damaged. Simple stagnation does not yield a poisonous substance, and consequently the release of the obstruction by operation is sufficient. When, however, strangulation has begun, the material above the obstruction should be removed, and if extensive damage exists a continued drainage through an enterostomy may be needed.

AN EXPERIMENTAL STUDY OF THE LATE GLOMERULAR LESIONS CAUSED BY CROTALUS VENOM.*

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PLATE 6.

In 1909 I described acute glomerular lesions produced in the rabbit by the use of rattlesnake venom.[1] These lesions, which followed the intravenous injection of carefully graded doses of venom, were either hemorrhagic or exudative in character and involved either the tuft or the capsular space or both. The tubular epithelium was either normal or showed minor changes only. As it seems probable, in view of the work of Flexner and Noguchi,[2] that the selective action on the glomerulus is to be explained by the action of the endotheliolytic body of venom, the lesion was considered as essentially a vascular nephritis with severe endothelial changes, and therefore as one offering an opportunity for attempting the experimental production of a chronic nephritis. During the past few years this possibility has been kept in mind, and from time to time in connection with various other phases of the studies of experimental nephritis a more or less intermittent study has been made of the lesions occurring after various lengths of time and after single and multiple injections of venom, in the hope of demonstrating chronic glomerular lesions.

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[1] Pearce, R. M., The Production of Edema. An Experimental Study of the Relative Importance of Renal Injury, Vascular Injury and Plethoric Hydremia, *Arch. Int. Med.*, 1909, iii, 422; An Experimental Glomerular Lesion Caused by Venom (*Crotalus adamanteus*), *Jour. Exper. Med.*, 1909, xi, 532.

[2] Flexner, S., and Noguchi, H., The Constitution of Snake Venom and Snake Sera, *Univ. Penn. Med. Bull.*, 1902–03, xv, 345.

The venom used in the experiments was the dried venom of *Crotalus adamanteus*.[3] It was dissolved in salt solution in the proportion of 0.25 of a milligram to one cubic centimeter, which was the usual initial dose. In the first series of animals fresh venom only was used, but as this frequently caused a rapidly fatal result, an old solution of venom which had been kept in the laboratory for a year was substituted. The older solution had lost much of its lethal power, that is, its neurotoxic and hemolytic powers were decreased, but its endotheliolytic power, as shown by the occurrence of albuminuria and the presence of exudation in the capsular space, had not been impaired. It was therefore used in the earlier treatment in doses of 0.25 or 0.5 of a milligram, rising gradually to two milligrams, and then followed by doses of 0.5 of a milligram of fresh venom at various intervals. The intervals between injections depended on the general condition of the animal and the amount of albuminuria. The initial injection was always followed by albuminuria and occasionally by hematuria or hemoglobinuria. When the albumin disappeared or had decreased greatly in amount, another injection was made, and as the periods of albuminuria lessened, the doses were increased. As sometimes a single injection caused a persistent albuminuria for weeks, while in other animals four to eight injections were given, the entire series represents the results of single and repeated injections. Despite the fact that some animals showed a tendency to immunization, it was impossible, even with the greatest care as to food and environment, to carry an experiment over a period of more than six to seven weeks. No cause could be found for this other than a progressive emaciation presumably dependent on the chronic intoxication.[4]

The results are based on the study of twenty-four rabbits, all of which received venom by intravenous injection.

Five animals died within four days after a single injection. Of these, one, which succumbed in six hours, presented the hemorrhagic, and four the typical exudative glomerular lesion of acute

[3] This venom was obtained through the kindness of Dr. Noguchi, of The Rockefeller Institute for Medical Research.

[4] In some instances the loss of weight was equal to 40 to 50 per cent. of the original weight, though usually it was about 25 per cent.

poisoning. One of the latter, dying on the third day, showed also an extreme degree of hyaline degeneration of the epithelium of the convoluted tubules.

Two animals died on the tenth day, after second and third doses respectively, with the usual acute lesions and no evidence of a reparative process. In each instance death followed an increase in the dose. Four died between the fourteenth and twentieth days. These represent one, two (2), and five injections; and all but one (representing two injections) showed evidence of repair of the glomerular lesion. The details of the experiments follow.

Experiment 2. Eighteenth Day Lesion; Multiple Injections.—A rabbit weighing 1,560 gm. received 0.5 mg. of venom (old solution) in the ear vein on October 2 and 4, and 1 mg. on October 6. As the injections caused little albuminuria the treatment was continued by using 0.5 mg. of fresh venom on October 11, 13, and 15. The last injections caused a pronounced albuminuria with hematuria. The animal died on October 19. Its weight was 950 gm. No gross lesions were found at autopsy.

Histological Examination.—The kidneys show well marked hemorrhagic and exudative lesions in the glomeruli; hyaline, granular, blood, and hemoglobin casts in both convoluted and collecting tubules; and granular degeneration of the epithelium of the convoluted tubules and loops of Henle.

In addition, however, to these acute lesions, the kidney shows distinct evidence of an attempt to repair the glomerular injury. Here and there it is possible to observe a penetration of the cells of the compressed glomerular tuft into the mass of hemorrhage lying either in the tuft itself or in the capsular space; less rarely is this seen when the capsule contains only serum and fibrin. The cells derived from the endothelium of the tuft are, for the most part, oval or elongated, with large deeply stained nuclei and scanty protoplasm which tends to take the basic stain. Despite the dense staining of the nuclei, a vesicular arrangement is frequently evident. Sometimes the cells, instead of penetrating the mass of hemorrhage, form a capsule about it, suggesting by their appearance, when they surround small masses, a giant cell formation. This proliferation is always from the tuft, never from the capsule (figure 1).

Experiment 7. Sixteenth Day Lesion; Multiple Injections.—A rabbit weighing 1,420 gm. received 1 mg. of venom (old solution) on October 4 and 6. The injections caused only a transient albuminuria, and therefore 0.5 mg. of fresh venom was given on October 11. This caused an albuminuria, increasing daily

in severity, with death on October 20. The weight of the animal after death was 870 gm.

Histological Examination.—The same lesions are found as in experiment 2, but with less marked evidence of glomerular proliferation.

Experiment 17. Eighteenth Day Lesion; Single Injection.—A rabbit received 0.25 mg. of fresh venom on October 26. This caused well marked albuminuria which persisted until death on November 14. Many of the glomerular tufts were compressed by hemorrhage into the capsule or distended by hemorrhage in the center of the tuft itself.

Histological Examination.—The cells of the compressed tufts show marked proliferation with a tendency for the new cells to invade the masses of hemorrhage. No typical mitotic figures are seen, but the character of both the nucleus and protoplasm indicates that these are young cells, and occasionally irregular masses of nuclear material suggest degenerated mitoses.

The period of twenty to thirty days is represented by three animals. Of these one, weighing 1,280 grams, died on the twenty-fourth day after a persistent albuminuria and a loss in weight of 520 grams. The injections were 0.5 of a milligram of venom on October 2 and 4, and one milligram on October 6, with death on October 26.

Microscopic examination shows that the acute lesions had subsided, the only evidence of their earlier presence being the presence of occasional casts and compressed masses of red cells in the glomerular spaces and tufts. About these masses definite proliferation of endothelial cells can be seen, and not infrequently endothelial cells are seen penetrating the hemorrhagic masses. Although no mitotic figures can be clearly distinguished, many of the compressed glomeruli appear to have an increased number of nuclei, and recall the glomeruli of subacute intracapillary glomerular nephritis as seen in man.

Of the other two animals of this group, one, receiving a single injection of 0.25 of a milligram of venom, died after twenty-nine days, and the other, receiving several injections, after twenty-six days. Albuminuria was persistent and marked in each case. Histological examination shows extensive granular degeneration of the convoluted tubules and many casts, but no glomerular lesions.

Seven animals survived for a period of one month or more. Two of these represent a single injection; the others, two to seven injections. The longest period of survival was thirty-eight days.

Albuminuria was persistent in all until death, but although all showed degenerative changes in the tubular epithelium, in none were glomerular changes prominent, and in only two were they even suggestive of reparative proliferation.

These observations indicate that in venom intoxication terminating in death at the end of five to six weeks the early acute glomerular lesion does not tend to become chronic; that is, it does not become transformed through proliferation and reparative processes into a subacute or chronic glomerulonephritis. Proliferation in the presence of an exudative (serum and fibrin) lesion was not seen; in the hemorrhagic lesion, proliferation of the endothelial cells of the tuft and the penetration of these cells into the masses of hemorrhage occurs, but this process is analogous to the organization of a red thrombus and is therefore not similar to the progressive proliferation of tuft or capsule seen in man. The attempt to produce, through the use of venom, a chronic glomerular lesion as the result of repair of acute lesions therefore fails, and with this failure disappears also the possibility of demonstrating experimentally that chronic glomerular lesions in man may have as their origin an injury caused by an endotheliolytic substance.

Several attempts to produce chronic lesions in the dog by repeated injections have likewise been unsatisfactory, owing to the fact that the toxic effect and the degree of hemolysis in this animal are much greater than in the rabbit. As the result of the study of the kidneys of five dogs receiving venom, it may be said that the lesion produced by small doses (0.25 to 0.5 of a milligram per kilo) is a moderate grade of degeneration of the tubular epithelium with cast formation. After the administration of larger amounts this degeneration is more severe, and to it is added the evidence of hemoglobin destruction; but seldom does one see in the glomerular capsule the exudate of serum, and never that of fibrin and red blood corpuscles, which is characteristic of the action of this poison on the rabbit's kidney. On the other hand, a peculiar hyaline change in the capillary loops of the glomerular tuft is a frequent lesion.

In no instance was it found possible to carry a dog for a long period of time. In attempts to accomplish this the smallest dose capable of causing albuminuria was administered, and this dose was

not given again until the albuminuria had disappeared. Despite this, it was not possible to carry an animal longer than two weeks, and at no time could evidence of reparative or subacute glomerular lesions be found.

SUMMARY.

The acute exudative glomerular lesion of the rabbit's kidney caused by crotalus venom does not lead to a subacute or chronic glomerulonephritis. The hemorrhagic lesion of the glomerular tuft may show a process of repair characterized by the ingrowth, into the hemorrhagic masses, of endothelial cells from the uninjured part of the tuft. This process is, however, more analogous to the organization of a red thrombus than it is to any form of glomerular lesion known in man, and can hardly serve as an experimental demonstration of the mode of development of a subacute or chronic glomerular nephritis.

On the other hand, crotalus venom causes a persistent albuminuria and extensive tubular degeneration and cast formation, with death, preceded by great emaciation, after five to six weeks.

EXPLANATION OF PLATE 6.

FIG. 1. Experiment 2. The glomerulus of a rabbit that died on the eighteenth day after repeated injections of crotalus venom. One half of the glomerulus is uninjured and is seen at the left of the figure; in the other half the hyaline area of an early hemorrhage is seen to be invaded by endothelial cells. Leitz oil immersion lens $\frac{1}{12}$; ocular 4.

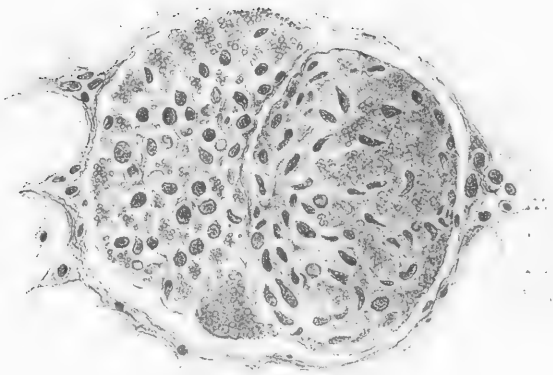

FIG. 1.

(Pearce: Lesions Caused by Crotalus Venom.)

CONCERNING VISCERAL ORGANISMS.*

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In previous articles[1] it has been shown that a fragment of heart pulsates normally for more than 100 days after its extirpation from the animal, and that connective tissue can be kept indefinitely proliferating *in vitro*. We have now observed strains of connective tissue cells that are still multiplying actively after more than sixteen months of life outside the organism. But up to the present only fragments of tissue have survived *in vitro*. Since the survival of entire organs outside of the body would undoubtedly have important physiological uses, I began in June, 1912, to develop a technique by means of which a system of organs could be made to live and functionate when separated from the other organs. Within a few months this technique has been sufficiently perfected to permit the separated organs being put to practical use for the study of several problems.

The method consists in removing aseptically the abdominal and thoracic organs of an animal and in preserving the organs in an incubator at a temperature of 38° C., while the lungs are being artificially ventilated. The operations were performed on both cats and dogs, but more often on cats. The animals were etherized and killed while they were still under the influence of the ether. Aseptic technique was employed and the same general rules were observed as in complicated thoracic or abdominal operations.

Two animals were used for each operation; the first one was prepared for transfusion, and from the second the viscera were taken.

* Received for publication, May 15, 1913.

[1] Carrel, A., *Jour. Exper. Med.*, 1912, xv, 516; Carrel, A., and Burrows, M. T., in Abderhalden, E., Handbuch der biochemischen Arbeitsmethoden, 1912, pt. v, 836; Ebeling, A. H., *Jour. Exper. Med.*, 1913, xvii, 273.

The first animal was etherized, the hair on the anterior part of the neck was removed with sodium sulphide, and the skin was washed with alcohol and sterilized with iodin. The neck was opened by longitudinal incision and one of the carotid arteries was dissected and opened after the circulation had been interrupted by a *serre fine*. The blood was washed out from the lumen of the vessel with Ringer solution, and the end of a glass cannula about thirty centimeters long was introduced and fixed by means of a circular ligature into the lumen of the artery. The cannula was not paraffined and no oil was used, on account of the danger of fat embolism of the coronary arteries of the second animal. Then the animal was kept etherized until it was time to transfuse the blood to the second animal.

The second animal was etherized, and the skin on the anterior part of the neck, thorax, and abdomen was shaved and sterilized by means of the technique previously described. The operation comprised four stages.

1. A long incision was made in the middle line from the upper part of the neck to the lower part of the abdomen, and the skin was dissected on each side of the line of incision. The trachea was dissected, and the esophagus was isolated, ligated, and cut under aseptic precautions. In some cases a catheter was introduced into the esophagus as far as the stomach, and fixed in such a way as to prevent any infection. Then the trachea was cut transversely and a curved glass cannula was inserted into its lumen and fixed by two circular ligatures. A gum catheter was introduced into the glass cannula and the trachea, as far as the bifurcation, according to the Meltzer-Auer method. The end of the catheter was connected with a foot bellows or a small electric blower.

2. The abdominal wall was opened on the middle line from the sternum to the pubis. The abdominal viscera were wrapped in a Japanese silk towel sterilized in vaselin, and were pushed up into the upper part of the abdomen. Branches of the mesenteric artery were ligated and cut, and the small intestine was prepared for section at a short distance from the large intestine. Then it was severed after having been ligated, and was cauterized with carbolic acid. The aorta and vena cava were exposed near the point of their bifur-

cation, ligated, and cut. The ureteral vessels were ligated and the ureters were severed. When the bladder had to be kept the ureters and their vessels were dissected as far as the posterior part of the bladder. The bladder itself was isolated from the surrounding tissues, the urethra was cut, after having been ligated, and the bladder was raised to the level of the kidneys. Then the aorta and vena cava were isolated from the abdominal wall and their posterior branches were tied. The peritoneum surrounding the kidneys was dissected and cut. All the small vessels uniting the aorta and the vena cava to the posterior part of the abdomen were also ligated and cut. The splanchnic nerves were cut and also the pillars of the diaphragm. Then the abdominal viscera, being completely separated from the abdominal wall, were wrapped in a Japanese silk towel. They remained united to the animal by the aorta and vena cava and their circulation was still active.

3. A transverse incision was made in the third or fourth intercostal space. The mammary arteries were clamped. Then the costal cartilages were cut on the right side of the sternum, and the thorax was widely opened. The diaphragm was severed at a short distance from the thoracic wall, and artificial respiration was established. It consisted of a current of air introduced into the trachea by means of a catheter and interrupted about sixteen times every minute. The lungs and the heart were protected by a Japanese silk towel, impregnated with vaselin. Then the innominate arteries or the carotid arteries were ligated and cut. The superior vena cava and the azygos vein were also tied and cut. After a few minutes the animal died, but the heart continued to pulsate. The vagus, sympathetic, and phrenic nerves were severed. Then the pleurae were cut on each side of the thoracic aorta. All the posterior branches of the thoracic aorta were clamped by means of a small Gentile hemostatic forceps and cut close to the thoracic wall. Generally the heart pulsated weakly and the blood pressure was low. The lungs and the abdominal organs were pale and no pulsations could be seen in the small arteries. In some experiments the heart stopped pulsating entirely.

4. Then the thorax and abdominal viscera, united by their blood vessels, were removed from the cadaver of the animal and placed

in a tray containing Ringer solution at a temperature of about 38° C., in such a manner that the lungs floated on the surface of the fluid and the heart was suspended underneath in the liquid. When this position was reversed and the heart was placed above the lungs the circulation was somewhat impaired. The temperature of the Ringer solution was maintained constant by means of an electric pad placed under the tray, or simply by the addition from time to time of Ringer solution at the right temperature. Ordinarily the heart still pulsated slowly and regularly, but the blood pressure was low and the appearance of the organs anemic. After a few minutes the blood pressure began to rise, and in a few cases became almost normal. Generally it remained low and sometimes the heart entirely ceased beating. Then a transfusion was made from the carotid artery of the first animal to the inferior vena cava or abdominal aorta of the visceral organism. When the pressure was very low or when the pulsations of the heart had completely stopped, the transfusion was made directly through the aorta, in order to reëstablish immediately a normal circulation through the coronary arteries. The heart started almost immediately to pulsate normally. As the condition of the heart improved the transfusion was made through the inferior vena cava. It was possible in that way to inject quickly a large quantity of blood into the visceral organism. Immediately after the transfusion the lungs became pink, the heart beat strongly from 120 to 150 times a minute, and the blood pressure often rose above normal. The abdominal aorta pulsated violently and strong pulsations could be seen in the arteries of the stomach, liver, kidney, intestine, and even of the ovaries. Peristaltic contractions of the stomach and of the intestines were observed. The spleen, which was bluish, assumed its normal appearance. After a few minutes all the viscera were apparently normal. Then a careful hemostasis of the posterior branches of the thoracic aorta was made and all the forceps were removed. It was important to ascertain that no hemorrhage, even one from a very small vessel, was taking place. If a few minutes after the transfusion the pressure was still above normal, a certain quantity of blood was allowed to flow from the lower part of the abdominal aorta. Then the appearance of the viscera exactly resembled that of a normal animal.

5. The visceral organism was placed in a tin box filled with Ringer solution, and covered with Japanese silk and protected by a glass cover. The tracheal and esophageal tubes were fastened to proper openings in the anterior part of the box. The intestine was pulled through a glass and rubber tube fixed in the posterior wall of the box. The end of the intestine was fixed by circular suture to the edge of the rubber tube, an artificial anus being made. The box was put into an incubator at a constant temperature of about 38° C. Artificial respiration was carried on by a current of air interrupted about twelve times a minute. The compressed air was furnished by means of an automatic electric apparatus pumping air into a tank, from which it was given to the animal under the proper pressure. Dr. Meltzer kindly examined the respiratory conditions in these experiments and showed me how a proper ventilation of the lungs could be obtained. His advice contributed in a large measure to the rapid success of the work. Water or food could be injected into the stomach through the esophageal tube. The urine could be collected from the bladder through a tube, but it was generally aspirated from the bladder with a needle, when samples of urine were taken for study. The feces and intestinal secretions were received outside of the box from the artificial anus.

It was then observed that during the hours following the operation the viscera had the same appearance as those of a living animal. The contractions of the heart and the circulation of the organs were apparently normal. Pulsations could be seen in the smaller branches of the mesenteric artery. The intestine emptied itself through the artificial anus by means of regular peristaltic contractions. When the intestine was empty, bile and intestinal juices were evacuated. In an experiment in which the stomach was full of meat at the time of death, digestion took place. Dr. Van Slyke found that at least 90 per cent. of the amino acids injected into the intestine were rapidly absorbed. There was also an abundant secretion of urine, which was collected into the bladder. After five or six hours hyperemia of the peritoneum of the intestine appeared. It seemed as though a peritonitis developed progressively, and in some cases the intestines became paralyzed after eight or nine hours, although their

circulation was still very active. Abundant hemorrhage could still be produced by section of a small branch of the mesenteric artery. Some of the visceral organisms died almost suddenly after three or four hours, but most of them were in a normal condition ten and even twelve hours after the death of the animal to which the organs belonged. The death of the organism was announced by some irregularities in the pulsation of the heart, which was also weaker. Then the heart stopped suddenly. In one experiment the death of the visceral organism occurred thirteen and one quarter hours after the death of the cat from which it was taken.

The technique of the operation can be modified in many different ways. The number and the nature of the viscera composing the organism were varied. The intestines, spleen, or kidney can easily be removed. It is also possible to exclude the liver from the circulation by ligation of the portal vein after a lateral anastomosis between the portal vein and the vena cava. The thyroid gland can be kept with its circulation or removed completely. The composition of the organism should be adapted to the problems which are to be studied.

The technique of preserving the organism is far from being definitely settled. The organs were generally kept in Ringer solution. Other kinds of fluid, such as serum or ascitic fluid, can also be used. I attempted to keep the viscera without any fluid at all in fine Japanese silk towels impregnated with vaselin. In this case the organism was put into a bag of very fine rubber, open at each end. Through one end was fixed the tracheal tube and to the other end the anal tube. The sac was then suspended in water. There was less danger of infection, but so far the results have not been as satisfactory as when the organs were placed directly in Ringer solution. In another experiment the entire thoracic cavity was kept. The operation consisted merely of a double amputation, through the abdomen and through the neck. A circular incision was made around the abdomen and the skin was dissected. The abdominal cavity was opened, and the intestine, aorta, and vena cava were ligated and cut. Then the anterior wall of the abdomen was sutured to the posterior wall and the abdominal cavity completely closed. The spinal column was cut

and the skin sutured. Afterwards the head was amputated and the stump was covered with the skin, leaving only an opening for the tracheal tube. A transfusion was made through the lower part of the aorta and the heart went on pulsating almost normally. Then the reduced organism was put into an incubator. It died of emphysema, because the air pressure became too high and produced rupture of the lung.

The technique will probably be progressively modified and adapted to the various problems of pathology, physiology, and biological chemistry, for the study of which the visceral organisms can be used.

THE CEREBROSPINAL FLUID IN SYPHILIS.*

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The results presented in this communication are based on the examination of 113 cases of syphilis in all stages of the disease, from the secondary eruption to general paresis. During the past ten months every case of syphilis admitted to the hospital has had lumbar puncture performed as part of the routine examination; previous to that time only cases which suggested infection of the nervous system were punctured.

TECHNIQUE.

Lumbar puncture is always performed with the patient lying on the right side. It has been found that a uniform position for all patients greatly increases the ease of the procedure, and special stress is laid on having the patient properly placed in the beginning. The troublesome headache sometimes seen after lumbar puncture is less frequent with the patient prone, than when the puncture is performed with the patient sitting up. The skin and underlying tissues at the site of puncture are infiltrated with a little 2 per cent. novocaine solution. This makes the procedure much less painful and patients rarely object to repeated punctures. The routine examination of the fluid is confined to four procedures,—estimation of pressure, cell count, globulin determination, and Wassermann reaction.

Pressure.—The pressure is estimated by allowing the fluid to run into a graduated manometer tube, with a bore three millimeters in diameter, and reading the height to which the fluid rises. This gives a figure which is, of course, relative only, but the normal variations

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in pressure are so large,—90 to 200 millimeters,—that a more accurate measure is unnecessary and only causes delay.

Cell Count.—The preparations should be made as soon as the fluid is removed, as the cells settle rapidly and adhere to the sides and bottom of the tube if the fluid is left standing; they may be counted at leisure, as long as sufficient time does not elapse for evaporation to occur. The counts are made with the pipette ordinarily used for leucocyte counts and the Thoma-Zeiss counting chamber. Two pipettes and two chambers are used in each estimation. For diluting fluid we use 10 per cent. acetic acid in distilled water. This is drawn to the "1" mark on the pipette, which is then filled to the "11" mark with spinal fluid. This gives a dilution of spinal fluid of nine in ten within the chamber. The fluid in the capillary portion of the tube is then blown out, and a drop of fluid from the chamber run on to the counting stage. The cover-slip is then placed on the slide and, while firm pressure is being applied, pushed across the stage. With this method Newton rings are easily obtained on all four sides, thus assuring the constant depth of the preparation. The cells on the whole ruled surface are then counted. This ruled surface in the Thoma-Zeiss chamber has an area of 9 square millimeters. As the chamber is only 1/10 of a millimeter in depth, the total count gives the number of cells in 9/10 of a cubic millimeter of the diluted fluid. The figure so obtained must, therefore, be multiplied by 10/9 to give the number of cells per cubic millimeter, and a further multiplication by 10/9 is required to correct the dilution; or, combining the two calculations, we can multiply by 100/81, that is approximately 10/8. The result gives the number of cells in 1 cubic millimeter of undiluted fluid. The two chambers should correspond closely. This method has proved entirely satisfactory, and accurate within 10 per cent. In considering the findings the standard of Dreyfus (1), based on 750 punctures, is used:

1 to 5 cells per c. mm.	normal.
6 to 9 cells per c. mm.	doubtful, border cases.
10 to 20 cells per c. mm.	slight lymphocytosis.
21 to 50 cells per c. mm.	moderate.
Over 50 cells per c. mm.	marked.

Globulin.—We have used the butyric acid method of Noguchi (2), which has been found entirely satisfactory. In some cases the colloidal gold method of Lange was also used, but in our hands this proved no more delicate and was less convincing than the Noguchi method. We have compared the Nonne-Apelt phase I reaction with the Noguchi butyric acid reaction in a number of fluids and have found the latter much the more delicate of the two. Where there is a marked excess of globulin, the results of the two reactions are quite parallel, but when the excess is slight, the phase I reaction fails, and fluids giving the degree of reaction classed by us as a ± reaction, that is, a faint haze with butyric acid, are with the Nonne-Apelt reaction entirely negative. The reagents used in the Noguchi reaction are butyric acid (Merck) diluted to 10 per cent. in 0.85 per cent. sodium chloride solution and a 4 per cent. solution of sodium hydrate. To 0.2 of a cubic centimeter of spinal fluid, 0.5 of a cubic centimeter of butyric acid is added and the mixture brought to a boil over a small flame; 0.1 of a cubic centimeter of 4 per cent. sodium hydrate is then added and the whole again heated to boiling. If carefully observed, the reaction can be delicately shaded and relatively quantitative readings made. The tubes should be watched carefully for the first ten minutes and read in half an hour and again in two hours. When possible several fluids should be done at the same time to obtain a comparison. In reading the Noguchi reaction we have adopted the following arbitrary standard:

—.....opalescence to very faint haze.
±.....faint haze to haze.
+.....fine granular precipitate.
++.....heavy granular or coarse flocculent precipitate.
+++.....very heavy flocculent precipitate.

Wassermann Reaction.—The introduction by Hauptmann and Hössli (3) in 1910 of the use of large quantities of spinal fluid in the Wassermann reaction entirely altered previous conceptions as to the value of this test in the examination of the spinal fluid. Previous to this it had been shown that of the four reactions used in determining the syphilitic nature of a disease of the nervous system.—Wassermann reaction in the blood, pleocytosis, globulin reac-

tion, and Wassermann reaction in the spinal fluid,—the latter was much the least frequently present. This was unfortunate as, when present, it was the most convincing of the four. The introduction of large amounts, five times as much fluid being used as is used in performing the reaction with serum, has made a positive Wassermann reaction in the spinal fluid one of the most constant findings in syphilis of the central nervous system. The test has, moreover, the great advantage that as yet it has never been found positive except where actual syphilitic infection of the central nervous system may logically be considered to have been present. Fraenkel (4) has checked by autopsy or operation the findings with these large amounts of fluid in thirty-six cases. In no case was a positive reaction obtained where syphilis of the central nervous system was not present.

In doing the Wassermann reaction we have used the anti-sheep hemolytic system with guinea pig complement. Daily titrations of complement have been made and the guinea pig serum has been diluted so that 0.5 of a cubic centimeter, the amount used in the test, contained two hemolytic units. Two units of hemolytic amboceptor have also been employed. The antigen used in most of the work was an alcoholic extract of human fetal liver, to which 0.25 per cent. cholesterin had been added. Latterly we have used an alcoholic extract of human heart to which 0.4 per cent. cholesterin had been added. The latter, which is a much more powerful antigen, was used with only a few of the fluids in this series. The findings in blood and spinal fluid are comparable, as reactions were always done with the same reagents. All the reagents were used in half the amount described in the original Wassermann reaction, giving a total volume of 2.5 cubic centimeters instead of the 5 cubic centimeters originally described. In some of the earlier fluids, only 0.1 of a cubic centimeter of fluid was used, but the majority were tested with 0.5, 0.4, 0.3, 0.2, 0.1, and 0.05 of a cubic centimeter (tables I to IX).[1] Twice the largest amount used in the test, that is, 1 cubic centimeter, was always used as anticomplementary control.

[1] As only half quantities of all reagents were used in performing the reaction, these amounts correspond to double the amount, or the 1.0 c.c., 0.8 c.c., 0.6 c.c. 0.4 c.c., etc., of Hauptmann, Nonne, Stühmer, Fraenkel, and others.

In no instance has the fluid been found to be anticomplementary in this amount, except in one purulent fluid from a case of epidemic meningitis. The following standard was adopted in reading the reaction:

++.....no hemolysis.
+±.....slight tingeing of supernatant fluid.
+.....50 per cent. of cells not hemolyzed.
±.....few cells not hemolyzed.
—.....complete hemolysis.

An attempt has been made to classify into groups, according to the clinical and pathological data obtainable, the patients whose spinal fluid findings are here reported. In some cases this has been easy and certain, but necessarily in a disease so complex and manifold as syphilis of the central nervous system, this grouping must in many cases be regarded as arbitrary. The following classification has been adopted: (1) secondary syphilis untreated; (2) secondary syphilis treated; (3) secondary syphilitic meningitis (including nerve relapses); (4) latent tertiary syphilis; (5) tertiary syphilitic meningitis; (6) syphilitic spinal spastic paralysis; (7) cerebrospinal syphilis; (8) cerebral syphilitic endarteritis; (9) tabes dorsalis; (10) general paresis.

SECONDARY SYPHILIS UNTREATED.

In 1903 Ravaut (5) reported on the examination of the spinal fluid in 116 cases of secondary syphilis. He found some degree of abnormality, either increased cells or increased protein content, in 67 per cent. Little attention was paid to these findings until the occurrence of the so called nerve relapses following treatment with salvarsan aroused fresh interest in the subject. In the past year several observers have examined the spinal fluid in cases of early secondary syphilis. All report frequent abnormal findings, and some give very high figures. Dreyfus (6) examined twenty-two cases of secondary syphilis and found changes in the spinal fluid in all, increased cells or protein in seventeen, and increased pressure in five. In a later paper (7) he states that 80 per cent. of cases of secondary syphilis show abnormalities in the spinal fluid. Wechselmann (8), basing his opinion chiefly on the results of the colloidal gold reaction of Lange, states that the majority of cases of syphilis show increased protein content of the spinal fluid. Boas and Lind (9) examined the spinal fluid in five cases of secondary syphilis and found counts of 9, 5, 3, 0, and 9. The Nonne-Apelt reaction was positive in two of the fluids; one of these had nine, the other no cells. The Wassermann reaction in the spinal fluid was negative in all (double amount used). Fraenkel (10) examined fifteen cases of early untreated

secondary syphilis. The fluid from five of these patients showed a definite lymphocytosis, in one case marked (272 cells per c. mm.); four other fluids showed counts between six and ten. The phase I reaction was positive in only one fluid. Five of the fifteen fluids gave a positive Wassermann reaction.

TABLE I.

Secondary Syphilis Untreated.

Name and case No.	Blood.	Cerebrospinal fluid.								
					Wassermann reaction in spinal fluid.					
	Wassermann reaction.	Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	0.5 c.c. (1.0 c.c.)[2]	0.4 c.c. (0.8 c.c)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c (0.1 c.c.)
J. L. 658	++	170	0	−	−	−	−	−	−	−
J. K. 636	++	140	1	−	−	−	−	−	−	−
G. S. 647	++	200	1	−	−	−	−	−	−	−
G. F. J. 717	++	175	1	−	−	−	−	−	−	−
J. O'D. 874	++	120	1	−	−	−	−	−	−	−
J. B. W. 741	++	170	2	−	−	−	−	−	−	−
L. M. 656	++	140	2	−	−	−	−	−	−	−
W. S. 649	++	170	2	−	−	−	−	−	−	−
P. C. 700	++	170	5	−	−	−	−	−	−	−
E. W. 882	++	180	1	−	−	−	−	−	−	−
W. B. M. 526	++	100	2	−	−	−	−	−	−	−
L. G. 885	++	85	4	−	−	−	−	−	−	−
J. C. 657	++		5	−	−	−	−	−	−	−
O. H. 683	++	200	5	−	−	−	−	−	−	−
M. F. 780	++		2	±	−	−	−	−	−	−
G. B. 644	++	170	1	±	−	−	−	−	−	−
H. R. 714	+	180	1	±	−	−	−	−	−	−
N. R. 665	+	120	7	±	−	−	−	−	−	−
J. H. 661	++		8	±	−	−	−	−	−	−
C. J. 884	+±	160	12	−	−	−	−	−	−	−
T. R. 157	++	180	12	±	−	−	−	−	−	−
L. R.	+±	270	13	±	−	−	−	−	−	−

We have examined the spinal fluid of twenty-two cases of early untreated secondary syphilis. Three showed cell counts of 12, 12, and 13, respectively; that is, slight but definite lymphocytosis. Two of these had also slight increase of globulin. Two with counts of seven and eight belong to the doubtful, border-line group; both of these had also, however, some increase of the globulin. Three cases with normal cell counts showed increased globulin. In only one case could the pressure be considered abnormal (270 millimeters). The Wassermann reaction was in every case positive in the blood and negative in the spinal fluid. Eight patients of

[2] See footnote 1, page 165.

the twenty-two examined, or 36 per cent., showed some abnormality of the spinal fluid. Of these eight patients, three have been lost track of, two have shown a normal spinal fluid after intensive treatment with salvarsan and mercury, one (case 157) has attended very irregularly and has had only three intravenous injections of salvarsan; his fluid six months later and again eighteen months later was entirely negative. One (case 644), who had increased globulin but no increase in cells has, in spite of two courses of neosalvarsan and one course of mercury injections, shown the same findings on both of two subsequent examinations, the globulin reaction being indeed more marked at the last examination. Another patient (L. R.) has, in spite of twelve intravenous injections of neosalvarsan and two courses of mercury injections, developed a definite meningitis with double optic neuritis; this is now, however, yielding to further treatment.

SECONDARY SYPHILIS TREATED.

All the patients in this group had been previously treated with salvarsan. These cases are patients who had received their treatment before routine lumbar punctures were made. With the exception of case 193, none of the patients showed symptoms referable to the nervous system. Case 193 was admitted in June, 1911, with active secondary syphilis and was given four intravenous injections of salvarsan, totaling 0.9 of a gram. The Wassermann reaction became negative in August, 1911, but was again positive in October, 1911. During November she had five intravenous injections of 0.2 of a gram of salvarsan. In January, 1912, she was admitted with a severe facial neuralgia of three weeks' duration. Physical examination showed nothing abnormal. Lumbar puncture yielded a spinal fluid with a cell count of 14; the globulin was negative. She was given 0.3 of a gram of salvarsan and the pain entirely disappeared within twelve hours and has not returned. She has since had 1.5 grams of salvarsan and 5.1 grams of neosalvarsan. Subsequent lumbar punctures showed on May 28, 1912, eleven cells, and on December 7, 1912, seven cells; there has never been any increase in the globulin. Table II shows that of ten cases of

TABLE II.

Secondary Syphilis Treated.

Name and case No.	Blood.	Cerebrospinal fluid.								
	Wassermann reaction.	Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	Wassermann reaction.					
					0.5 c.c. (1.0 c.c.)[8]	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.5 c.c. (0.1 c.c.)
P. M. 433	+±	80	6	−	−	−	−	−	−	−
H. H. C. 37	−		4	−	−	−	−	−	−	−
A. W. C. 13	−		4	−	−	−	−	−	−	−
W. S. F. 39	−	120	3	−	−	−	−	−	−	−
A. F. 71	−	170	5	−	−	−	−	−	−	−
R. M. H. 18	−	170	5	−	−	−	−	−	−	−
R. H. 586	±	160	3	−	−	−	−	−	−	−
J. C. 613	++	160	6	−	−	−	−	−	−	−
C. S. D. 193	−		14	−	−	−	−	−	−	−
H. G. 73	++	180	1	−	−	−	−	−	−	−
Tertiary Syphilis Treated.										
J. H. T. 167	+	140	1	−	−	−	−	−	−	−
J. P. H. 642	++	110	0	−	−	−	−	−	−	−
F. L. 70	±	140	1	−	−	−	−	−	−	−
B. S. 204	+	140	1	−	−	−	−	−	−	−
A. D. 1,093	++		4	−	−	−	−	−	−	−
J. Z. 654	++	70	1	+	−	−	−	−	−	−

treated secondary syphilis, one, or 9 per cent., showed an abnormal fluid. This patient showed also clinical evidence suggesting involvement of the nervous system. Two showed doubtful or border-line counts with no increase of globulin.

SECONDARY SYPHILITIC MENINGITIS.

This group is made up of patients who had an evident meningitic involvement in the active secondary period; that is, in the first year of the disease. They should, perhaps, be included in class 2 (secondary syphilis treated), as all had had more or less salvarsan. Only four of these patients, however, belong in the group of patients with secondary syphilis treated by us and still under observation. The other four patients were admitted because involvement of the central nervous system was suspected. Moreover, none were discovered in the course of routine lumbar puncture, but all were examined because a lesion of the central nervous system was suspected. For these reasons they were classed separately.

[8] See footnote 1, page 165.

TABLE III.

Secondary Syphilitic Meningitis.

Name and case No.	Wassermann reaction in blood.	Cerebrospinal fluid. Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	Wassermann reaction. 0.5 c.c. (1.0 c.c.)[4]	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c. (0.1 c.c.)	Time since chancre.	Time since first nervous symptom.	Treatment before first nervous symptom.	Treatment before lumbar puncture. Salvarsan.	Mercury.
D. M. T. 187	−		575	++	±	±	−	−	−	−	6 mos.	3 mos.	Salvarsan 0.7 gm. Mercury 4 injections	0.7 gm.	8 inj.
P. H. 144	−		132	+	(±)[5]	(−)	(−)	(−)	−		9 mos.	7½ mos.	Mercury *per os* 2 weeks	1.8 gm.	4 inj.
M. H. 404	−	135	110	±	++	+±	+	±	−	−	8 mos.	(?)	None	2.8 gm.	4 inj.
A. B. R. 29	−	230	215	±	++	++	+±	−	−	−	18 mos.	17 mos.	None	5.4 gm.	33 inj.
C. W. W. 392	++	225	1074	++	(++)	(++)	(++)	(++)	++		5 mos.	1 mo.	Salvarsan 1.5 gm. Mercury 12 injections	1.5 gm.	12 inj.
W. E. 655	+	190	190	±	++	++	+±	−	−	−	6 mos.	5 mos.	None	1.0 gm.	
F. S. 875	+	200	181	++	++	++	+	−	−	−	11 mos.	10½ mos.	None	0.5 gm.	Mercury *per os* 4 mos.
F. W. J. 730	+	210	30	+	−	−	−	−	−	−	7 mos.	6½ mos.	None	0.5 gm.	8 inj.

[4] See footnote 1, page 165.

[5] The readings in brackets indicate that at the time of the first examination of the fluid only 0.1 c.c. was used, the bracketed readings having been obtained on subsequent examination after the introduction of the use of larger quantities.

These cases include four typical examples of the so called neuro-relapse with paralysis of cranial nerves (cases 187, 392, 655, and 875). The clinical picture in these patients with secondary syphilitic meningitis varies greatly. In a previous paper (11) the relative insignificance of the clinical symptoms and signs which may be present in patients yielding such fluids has been pointed out. They may, on the other hand, show most extreme evidence of their nervous involvement. As will be seen by reference to table III, the spinal fluids of these patients show very consistent findings, more so than any other type of syphilis of the nervous system. Case 730 alone is irregular. We find, almost without exception, high cell counts, this condition yielding, as a rule, the most marked pleocytosis of any syphilitic condition of the nervous system. In our cases the globulin varied from a heavy flocculent precipitate (++ reaction) to a faint haze (± reaction), but was positive in every case. Both the globulin and the number of cells paralleled closely the clinical condition, being most increased in the severe cases. The Wassermann reaction in the spinal fluid was positive with 0.5 of a cubic centimeter of fluid in every case but one, while with 0.1 of a cubic centimeter it was positive in only one. The Wassermann reaction in the blood was positive in four and negative in four cases, being apparently determined by the amount of previous treatment. These patients have responded satisfactorily to treatment, and all those who have been treated and followed (six) are now free from symptoms and all but one have normal spinal fluids. They are being kept under constant treatment with mercury and salvarsan in spite of the negative findings.

It is interesting to compare the findings in our cases of treated secondary syphilis with those of Altmann and Dreyfus (12), who examined the spinal fluid in eight cases of primary and fifty-six cases of untreated secondary syphilis. Two of the eight fluids from their primary cases showed cell counts of eight and thirteen respectively; both of these gave a positive phase I reaction. Seven of the fluids from the fifty-six patients with secondary syphilis, examined by them, had cell counts of between 75 and 500 cells per cubic millimeter; all the seven fluids showed also a marked increase

in the globulin and a positive Wassermann reaction. The Wassermann reaction in the spinal fluid was positive in eleven of the fifty-six cases; only once was it positive without an accompanying marked increase in cells and globulin. Many of the other fluids showed slighter changes, 66 per cent. in all of the fluids from fifty-six untreated secondary cases showing some increase in cells or globulin.

As will be seen by reference to table I, the number of our patients with untreated secondary syphilis who showed abnormalities in the spinal fluid is much smaller than that found by Altmann and Dreyfus, and the changes are much less marked. The discrepancies may be due to the fact that nearly all the patients were in the very early secondary stage. Altmann and Dreyfus found the marked changes,—cell counts of over 100, strong globulin reaction, and positive Wassermann reaction in the spinal fluid,—only in the later secondary cases. Table III shows that at this period of the disease we also had a number of patients whose spinal fluids showed similar findings. If the question of previous treatment with salvarsan be omitted, our findings in forty cases of secondary syphilis, cell counts of 30 to 1,074 in eight, positive Wassermann reaction in the spinal fluid in seven, and slight increase in cells or globulin in eleven, are comparable to those of Altmann and Dreyfus. From these figures it would appear, contrary to what is commonly held, that changes in the spinal fluid are not more frequent in treated than in untreated patients. These findings, meaning, as they almost certainly do, an infection of the central nervous system with *Spirochæta pallida*, emphasize the frequency of involvement of the central nervous system in syphilis and how early in the disease this invasion occurs.

LATENT TERTIARY SYPHILIS (WITHOUT NERVOUS MANIFESTATIONS).

Six patients were examined (table II, part 2) and in only one was any abnormality in the spinal fluid detected. This patient was admitted with lobar pneumonia. She denied syphilis, but gave a history of repeated miscarriages. The Wassermann reaction in the blood was strongly positive. Her spinal fluid showed no increase in cells, but gave a well marked globulin reaction.

TABLE IV.

Tertiary Syphilitic Spinal Meningitis.

Name and case No.	Blood.	Cerebrospinal fluid.								
	Wassermann reaction.	Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	Wassermann reaction.					
					0.5 c.c. (1.0 c.c.)[6]	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.5 c.c. (0.1 c.c.)
V. H. 565	+±	220	124	+++	++	++	++	++	++	±
I. P. 660	+	195	15	−	−	−	−	−	−	−
F. Z. 691	−	170	24	+	−	−	−	−	−	−

TERTIARY SYPHILITIC MENINGITIS.

In this group (table IV) were placed those patients whose symptoms and signs could be explained by inflammatory thickened meninges pressing on nerve roots, and in whom no evidence of lesions of the actual nervous tissue of the cord could be obtained. The findings in the spinal fluid in the three cases were varied.

TABLE V.

Syphilitic Spinal Spastic Paralysis.

Name and case No.	Blood.	Cerebrospinal fluid.								
	Wassermann reaction.	Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	Wassermann reaction.					
					0.5 c.c. (1.0 c.c.)[7]	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c (0.1 c.c.)
R. W. 94	±		5	++					−	
G. L. 603	++	260	36	++	++	++	++	++	±	−
J. McE. 342	++	165	10	++					−	
B. F. W. 933	+±	120	20	+	−	−	−	−	−	−

SYPHILITIC SPINAL SPASTIC PARALYSIS.

Case 603 (table V) is a typical example of the type of spastic paraplegia first differentiated and described by Erb, and especially studied by Oppenheim and by Nonne. The condition consists of a slowly developing spastic paralysis of the lower extremities, with spastic gait, muscular weakness, increased tendon reflexes, and

[6] See footnote 1, page 165.

[7] See footnote 1, page 165.

bladder disturbance. The upper extremities are normal and there is no change in mentality, cranial nerves, or pupils, and no disturbance of sensation. The other three patients present almost an identical clinical picture, but in them the onset was in every case sudden. In these cases the lesions are usually considered to be chiefly vascular, and the findings in the spinal fluid,—low cell count and strong globulin reaction,—support this view.

CEREBROSPINAL SYPHILIS.

There is probably no pathological condition the signs and symptoms of which are so manifold and varied as cerebrospinal syphilis. This is to be expected from the variation in site and character which the process exhibits. The cerebrospinal fluid, on the other hand, shows a fairly constant picture (table VI), the greatest variation being in the magnitude of the cell increase. Almost every case shows a strong globulin reaction and a positive Wassermann reaction in the spinal fluid. The difference in the number of positive Wassermann reactions in the spinal fluid when 0.5 of a cubic centimeter is used, instead of 0.1 of a cubic centimeter, is well illustrated in this condition. With 0.1 of a cubic centimeter, only seven cases out of twenty-one, or 33 per cent., were positive, while with 0.5 of a cubic centimeter, fifteen out of sixteen fluids examined, or 94 per cent., gave a positive reaction. Hauptmann (13) examined forty-four cases of cerebrospinal syphilis which were negative with the smaller amount of fluid; of these forty-two were positive when larger amounts of fluid were used. One or two of the cases, whose spinal fluid findings are given in table VI, will be given in more detail, as they illustrate the slight signs and symptoms which may be shown by patients whose spinal fluid shows them to be victims of extensive active disease. Case 913 had complete loss of reaction to both light and accommodation in both pupils. This was of eleven years' duration. He had no symptoms beyond disturbed vision, and except for the pupil change and the absence of one tendon Achilles reflex, his physical examination was negative. Case 605 had a similar paralysis of accommodation in one eye of two years' duration; he has had no symptoms

TABLE VI.

Cerebrospinal Syphilis.

Name and case No.	Blood. Wassermann reaction.	Cerebrospinal fluid. Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	Wassermann reaction. 0.5 c.c. (1.0 c.c.)[8]	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.5 c.c. (0.1 c.c.)	Years since chancre.	Secondaries.	Time since onset of nervous symptoms.
M. C. 913	++	210	13	++	++	++	++	+	−	−	20	Slight	11 yrs.
F. K. 605	+±	160	60	++	++	++	++	++	−	−	Denied	Denied	2 yrs.(?)
H. G. 1,199	−	100	58	+	++	++	++	++	±	−	Denied	Denied	3 yrs.(?)
J. H. R. 154	++		63	+++					−		Denied	Denied	2 yrs.
L. G. 888	+	150	53	+	++	++	++	+	−	−−	11	Moderate	9 mos.
W. S. 1,118	++	230	183	++	++	++	++	++	++	−	6½	None	6 yrs.(?) 1 yr.
M. P. 794	−	120	40	+	(++)[9]	(++)	(++)	(+±)	−	−	3½	Moderate	1½ yrs.
J. R. W. 887	±		135	+++	++	++	++	++	++	++	15	Moderate	2 yrs.
H. B. 934	+	240	233	+	+	−	−	−	−	−	13	Moderate	4 mos.
D. J. W. 806	−	260	98	+++	+	±	±	−	−	−	4	Moderate	1 yr.
R. B. 1,201	−	230	22	++	++	++	++	++	−	−	5½	Moderate	1 yr.
L. C. 562	−	260	65	+	++	++	++	++	++	±	2	Moderate	1 wk.
A. DeL. 153	−	70	35	++					−		17	(?)	13 yrs.
V. der H. 52	++	220	5	++					−		7	Moderate	4 mos.
R. O. 168	++	165	2	+					±		Denied	Denied	1 yr.(?)
F. B. 345	++		10	+					−		20 mos.	Moderate	7 mos.
U. C. B. 1,032	±	140	50	+	++	++	++	++	−	−	Denied	Denied	1 yr.(?)
L. B. 720	++		560	++	++	++	++	++	++	++	Congenital		1 wk.
C. G. 1,304	−	110	90	++	++	++	+±	±	−	−	25	Moderate	3 yrs.
J. M. 593	++	100	38	++	++	++	++	++	++	+	7	Moderate	2 yrs.
L. H. 937	−	230	5	±	−	−	−	−	−	−	31	Moderate	18 yrs.
Positive, per cent.....	62		86	100	94	88	88	81	33	25			

Total No. of cases 21.

[8] See footnote 1, page 165.

[9] The readings in brackets indicate that at the time of the first examination of the fluid only 0.1 c.c. was used, the bracketed readings having been obtained on subsequent examination after the introduction of the use of larger quantities.

beyond two momentary attacks of slight dizziness, and his physical examination was otherwise entirely negative. Cases 154 and 1,199 are similar cases of isolated pupil disturbance; they have, now, however, developed other signs of nervous involvement. Case 888 had no symptoms except "general nervousness" and some chronic gastric distress; his physical examination was entirely negative. Case 887 had headaches of eighteen months' duration and deafness in one ear; his physical examination showed beginning optic atrophy and nerve deafness in the affected ear; he had visited numerous eye and ear dispensaries, but had been given no antisyphilitic treatment; his headache disappeared within forty-eight hours after starting treatment. Case 1,032, a man aged thirty-eight years, noticed in December, 1911, some unsteadiness of station. In February, 1912, he was taken with sudden weakness of the lower extremities which gradually increased until June, 1912, when he became confined to bed. He entered the hospital on November 29, 1912, with a complete flaccid paralysis of the lower extremities, weakness of the muscles of the back, tingling of the fingers, and some weakness and huskiness of voice. He showed slightly unequal contracted pupils which reacted sluggishly to light and accommodation, areas of anesthesia to light touch over the trunk and lower extremities, and a complete flaccid paralysis of the lower extremities, with loss of all tendon reflexes. There was slight atrophy of both lower extremities with marked muscular hypotonus, fibrillary twitching, and loss of myotatic irritability. The reaction of degeneration was not present. This is apparently an example of syphilitic anterior poliomyelitis. Nonne (14) has collected a number of such cases from the literature and reports three cases of his own. The sensory changes in this case, however, remove it from the pure cases of this type, in which evidence of lesions in the anterior horn alone is found. Case 720 is similar. This child was sent in as a case of acute anterior poliomyelitis; the true nature of the disease was only suspected when it continued to progress. Under specific treatment improvement rapidly occurred.

CEREBRAL SYPHILITIC ENDARTERITIS.

In this group (table VII) we have placed the cases whose clinical and spinal fluid findings suggest that their symptoms are

due to vascular disease rather than to a syphilitic infection of the actual nervous tissue. These patients have not, in all probability, a syphilitic infection of the central nervous system, any more than has a case of aortitis, the disturbance of the nervous system in them being entirely secondary and accidental, dependent only on impaired nutrition due to vascular disease. The clinical diagnosis has since

TABLE VII.

Cerebral Endarteritis (?). Indefinite Cerebral Symptoms. Negative Findings.

Name and case No.	Blood.	Cerebrospinal fluid.								
	Wassermann reaction.	Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	Wassermann reaction.					
					0.5 c.c. (1.0 c.c.)[10]	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c (0.1 c.c.)
D. T. 568	−		1	+	−	−	−	−	−	−
M. L. S. 427	−	210	1	−	−	−	−	−	−	−
M. C. C. 662	−	170	[illegible]	−	−	−	−	−	−	−
W. S. H. 746	−	120	4	−	−	−	−	−	−	−
J. M. S. 422	++	140	2	−	−	−	−	−	−	−
B. T. 528	−	150	3	−	−	−	−	−	−	−

been confirmed in one case at autopsy. Nonne (15) has called attention to the absence or trivial character of the spinal fluid findings in this type of case. Clinically they showed indefinite cerebral symptoms,—headache, dizziness, "nervousness,"—in short, the ordinary symptoms of cerebral arteriosclerosis. Physical examination in these patients was negative, and the spinal fluid showed no abnormality, except, in one of our cases, an increase of globulin.

TABES DORSALIS.

The examination of the spinal fluid in this condition yields information of great value. By this procedure an early diagnosis can be confirmed, and a valuable index of the result and efficiency of subsequent therapy is obtained. Moreover, no adequate conception of the course of the syphilitic process can be reached until all available sources of information have been investigated in each case, and until the history, physical signs, and findings in the blood and spinal fluid have been analyzed and correlated.

[10] See footnote 1, page 165.

TABLE VIII.

Tabes Dorsalis.

Name and case No.	Blood. Wassermann reaction.	Cerebrospinal fluid. Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	Wassermann reaction. 0.5 c.c. (1.0 c.c.)[11]	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c. (0.1 c.c.)	Years since chancre.	Secondaries.	Years since onset of tabes.
F. H. P. 1,097	++	160	225	++	++	++	++	++	++	++	8	Slight	1½
J. J. McV. 1,136	++	120	143	+	++	++	++	++	−	−	18	Severe	7
C. M. 485	++	220	130	++	++	+±	+±	±	−	−	19	Denied	1½
C. L. V. 85	++	120	125	++	(++)[12]	(+)	(±)	(−)	−		16	Denied	6
A. T. 1,177	−	180	114	++	++	++	++	+±	−	−	8	Slight	1
O. K. 1,159	++	120	112	++	++	++	++	++	+±	−	12	Slight	4
O. W. A. 213	++	180	110	−	(++)	(++)	(++)	(++)	++		9	Moderate	2½
C. M. A. 1,111	±	180	92	±	++	++	++	++	+	−	17(?)	Moderate	11
W. L. S. 113	++	60	72	++	(++)	(++)	(+)	(−)	−		23	Moderate	7
A. D. 1,140	++	180	57	++	++	++	++	+±	−		14	Moderate	1½
M. B. 517	−	200	55	+	±	±	−	−	−	−	Denied	Denied	3
R. d'H. 674	++	130	55	+	++	++	++	++	±	−	Chancroids 7	Denied	3
S. C. 560	+	200	50	++	+	±	−	−	−		14	Denied	Tabes optica 1
R. S. P. 1,186	++	130	47	++	++	++	++	++	−	−	Denied	Denied	4 mos.
O. J. L. 836	−	100	45	±	−	−	−	−	−	−	15	Marked	11
A. N. P. 903	++	180	44	±	++	++	++	++	−	−	16	Very slight	10(?)
H. S. R. 1,042	−	230	42	−	++	++	++	++	++	−	Denied	Denied	3
C. H. L. 557	−	40	42	++	(++)	(++)	(++)	(++)	++		30	Denied	10
F. B. 893	+	120	40	±	+	±	−	−	−	−	Chancroids 13	Denied	2
H. G. G. 675	−	160	35	±	++	++	++	+	−	−	Denied	Denied	2½
J. A. B. 1,141	++	180	33	+	++	++	+	+	−	−	32	Denied	2
J. J. S. 559	+	180	30	+	+	±	±	−	−	−	8	Denied	1½
O. F. 152	++	75	27	++	(++)	(++)	(++)	(++)	−	(−)	10	Denied	3½
W. P. K. 980	−	50	16	+	++	++	++	−	−	−	12	Denied	6

Name and case No.	Blood.	Cerebrospinal fluid.									Years since ancre.	Second-aries.	Years since onset of tabes.
	Wassermann reaction.	Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	Wassermann reaction.								
					0.5 c.c. (1.0 c.c.)[11]	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c. (0.1 c.c.)			
P. J. F. 555	−	130	14	+	−	−	−	−	−	−	8	Mild	1
C. L. B. 848	−	100	9	−	+	−	−	−	−	−	8(?)	Denied	4(?)
J. F. E. 679	±		9	+	++	++	−	−	−	−	23	Moderate	12
B. A. 80	−		8	±					−		29	Denied	13
B. F. Y. 847	±	160	7	+	−	−	−	−	−	−	15	(?)	3 to 7
J. W. 524	++	150	3	−	−	−	−	−	−	−	10	Denied	$1\frac{1}{2}$
Positive, per cent.....	66		97 83	87	86	83	69	55	23	4			

Total number of cases 30.

[11] See footnote 1, page 165.

[12] The readings in brackets indicate that at the time of the first examination of the fluid only 0.1 c.c. was used, the bracketed readings having been obtained on subsequent examination after the introduction of the use of larger quantities.

Thirty patients with tabes dorsalis have been under observation and treatment in the hospital. The spinal fluids of all but one of these patients showed an increase in cells. Four cases showed, however, cell counts between six and nine. These must be looked upon as border-line counts and cannot be regarded as true lymphocytosis. Two of these four patients (cases 847 and 848) had each had two injections of salvarsan at other hospitals before admission. If we omit these doubtful cases, twenty-five, or 83 per cent., of the fluids examined showed a true lymphocytosis. The globulin reaction was positive in twenty-six cases, or 87 per cent. The Wassermann reaction in the spinal fluid, with 0.1 of a cubic centimeter of the fluid, was positive in seven and negative in twenty-three, giving 23 per cent. of positive reactions; two of the seven positive reactions were only weakly positive. With 0.5 of a cubic centimeter of fluid the reaction was positive in twenty-four and negative in four, giving 86 per cent. of positive reactions. It will thus be seen that lymphocytosis, increased globulin, and a positive Wassermann reaction in the spinal fluid occur with practically equal frequency. One patient with an outspoken tabes (case 524) had a perfectly normal spinal fluid.

The Wassermann reaction in the blood is less frequently present than the other three reactions. In this series twenty patients, or 66 per cent., had a positive reaction in the blood. The results of the Wassermann reaction in this series of thirty patients (66 per cent. positive in the blood, 86 per cent. positive in the spinal fluid), are in striking agreement with those of Hauptmann (13), who, in a similar series of tabetics, found 66 per cent. positive in the blood, and 87 per cent. positive in the spinal fluid. One of us (Swift (16)) in a previous series of tabetics also found 66 per cent. of positive reactions in the blood.

A striking fact brought out in table VIII is the trifling character of the secondary manifestations of syphilis in the majority of patients subsequently developing tabes dorsalis. Sixteen of the thirty patients, or more than 50 per cent., denied all knowledge of secondary manifestations. This is a rule to which attention has been called by numerous observers, and has been especially emphasized by Mott (17). The figures are more striking if one

compares the findings in cerebrospinal syphilis (table VI), where only five of the twenty-one patients denied secondaries. Moreover, three of the five cases of cerebral syphilis denying secondaries (cases 605, 1,199, and 154) are patients with an ophthalmoplegia as practically the sole evidence of their disease,—a class of case which, Nonne (14) states, almost always develops later into either tabes or paresis.

GENERAL PARESIS.

Only three cases of general paresis have been observed. Two of these were very early cases (Nos. 42 and 883). All three showed a positive Wassermann reaction in the blood, moderate or slight pleocytosis, a strong globulin reaction, and an intensely strong posi-

TABLE IX.

General Paresis.

Name and case No	Blood.	Cerebrospinal fluid.								
					Wassermann reaction.					
	Wassermann reaction.	Pressure in mm.	Cell count per c. mm.	Noguchi globulin reaction.	0.5 c.c. (1.0c.c.)[13]	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.5 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c. (0.1 c.c.)
A. 42	+	145	16	+++	++	++	++	++	++	++
M. N. 883	++	90	46	++	++	++	++	++	++	++
H. L. P. 1,172	++		9	++	++	++	++	++	++	+

tive Wassermann reaction in the spinal fluid. In two cases the fluid gave complete fixation of complement with 0.006 of a cubic centimeter of spinal fluid. The occurrence of a positive Wassermann reaction with such small amounts of spinal fluid, 0.05 of a cubic centimeter and less, seems, in our experience and that of others, to be of value in attempting to differentiate cases of general paresis from the gummatous and vascular forms of cerebral syphilis. Nonne and Holzmann (18) are of the opinion that these findings are also significant in attempting to recognize cases of taboparesis. Our experience in a limited number of cases tends to confirm this opinion. Only one of our tabetics (case 1,097) gave a positive reaction with this amount of fluid, 0.05 of a cubic centimeter. This patient had a definite change in personality and mental deterioration, and is almost certainly a case of taboparesis.

[13] See footnote 1, page 165.

In conclusion we should like to emphasize the value of the frequent use of lumbar puncture in the care and study of syphilis. The procedure is simple, and the information obtained of extreme value. In considering prognosis and the amount of treatment likely to be required, the examination of the spinal fluid for possible abnormalities in the secondary stage of the disease is of the utmost importance. The diagnosis of any syphilitic condition of the central nervous system is not complete without an examination of the spinal fluid, and in the treatment of these conditions repeated examinations of the fluid furnish the best guide to therapy. A negative Wassermann reaction in the blood is no evidence that the nervous system is not affected and every patient with increased cells, globulin, or a positive Wassermann reaction in the spinal fluid needs active antisyphilitic treatment. No patient who has been infected with syphilis can conscientiously be discharged as cured, until the spinal fluid has been examined and found normal, even though he remains free from signs and symptoms and has a persistently negative Wassermann reaction in the blood.

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THE GROWTH OF TISSUE IN ACID MEDIA.*

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PLATE 7.

It has often been shown that the higher animals are extremely sensitive to changes in the reaction of their circulating fluids, and that if the blood be made acid death results. There seems to be a general belief that the cells themselves are unable to withstand an acid reaction of the medium about them. The truth of this belief can now be tested by the use of tissues growing *in vitro*.

In the course of some work with the cells of a chicken sarcoma proliferating *in vitro* I was led to add blue litmus to the plasma medium, and observed that a marked acid change accompanied cell growth. All the tissue fragments were at first stained blue and those which remained inert did not lose this color; but the others which were destined to proliferate became violet or pink within a few hours, often indeed before any sprouting out of cells was observable; and any considerable degree of growth always resulted in a focal pink change in the medium. The mere elaboration of acid by growing tissues is in itself no cause for remark. But the reaction with litmus means that a considerable amount of acid is produced, since this indicator when in plasma is relatively insensitive, owing to the high combining power for acid of the proteins. And that the cells should be capable of further active growth in the medium which they have rendered acid deserves attention and calls for further observations.

Chicken plasma, chicken sarcoma, and the heart muscle and large blood vessels of embryo chicks have been used for the present experiments. The ordinary, hanging-drop plasma preparation was found

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to be unsuitable since it presents too thin a layer for the proper observation of color changes. Instead small Petrie dishes have been used and the tissue bits distributed in a layer of plasma one to two millimeters thick. With pure azolitmin the results are not good, and with pure litmus (Merck's reagent) they are no better than with the watery solution obtained from Kahlbaum's cube litmus and used for tinting litmus milk. This solution as prepared by adding 250 cubic centimeters of water to 500 grams of litmus, heating to 80° C. for one hour, and filtering, is distinctly though only slightly alkaline. It contains numerous impurities including carbonates. When added to chicken plasma, as yet unclotted, in the proportion of one part to ten it colors the medium a deep blue. Such a medium is better than a violet one because the color changes are sharper.

The findings with the chicken sarcoma and with non-neoplastic connective tissue differ only in intensity. The production of acid by the chicken sarcoma in quantities sufficient to change the reaction of the medium is prompt and marked, as is the sarcoma's growth. In one instance where eight or ten small fragments of the tumor grew in a dish with 0.8 of a cubic centimeter of plasma mixture, the diffuse pink change in the medium at the end of two days' incubation was found by test to be equivalent to that brought about in a similar bulk of the unclotted medium by 0.12 of a cubic centimeter of N/10 hydrochloric acid. Fragments of tumor or of late embryos (twenty days) placed in the tinted plasma are all at first colored blue, and only those which start to grow show the change to violet and pink. The color differences between active and inert tissue bits are very striking in dishes where some fragments grow and others perish (figure 1). They constitute a good macroscopic index to proliferation. The growing tissue itself appears as a whitish, unstained zone, or halo, in a pink medium. Material from very young embryos, which survives practically *in toto* the transfer to the plasma medium, likewise remains unstained, and some of the visceral tissues from such a source appear to affect the reaction of the plasma medium very little in their growth.

Tissues grow excellently in a medium tinted with methyl orange, but the acids they produce do not affect this indicator,—presumably because they are organic. With congo red a slight violet change is

sometimes observed in the zone of proliferation, but the reaction is only definite in the case of cells into which the stain has penetrated. These are practically always cells distended with fat droplets and far gone in degeneration. Their cytoplasm may be colored a bright violet. Dead tissue fragments stain red usually, but growing tissue is unaffected by the stain and is seen as a pale halo in the midst of the red medium. The occasional violet tinting of isolated cells cannot be due to free carbon dioxide, for though congo red reacts to it in water this does not happen when the gas is bubbled through unclotted plasma tinted with the indicator.

A striking fact with regard to the elaboration of acid in the plasma medium, and one which must be of much importance for tissue cultures is the small extent to which it diffuses. It is relatively unaffected, as regards neutralization, by the amount of plasma beyond the zone of growth, and this even when the plasma web has been thinned by dilution. When growth is stopped by placing the preparation in the ice-chest, and alkaline plasma is present beyond the zones of growth, neutralization gradually occurs in the latter, but so slowly that it may be incomplete at the end of forty-eight hours. Under conditions *in vitro* which do not permit of an artificial circulation the growing tissue finds itself almost at once in an acid medium. When growth is prolonged by washing the tissue in Ringer solution and reimplanting it in fresh plasma, its alkaline reaction is temporarily restored, only to disappear as growth begins anew. The more rapid the growth of a tissue *in vitro* the less are the opportunities for its products of metabolism to be diluted by diffusion, and so the more unfavorable the conditions under which its growth must continue. This circumstance probably accounts to a considerable degree for the difficulties which Lambert[1] has experienced in keeping rat tumor alive *in vitro*. Similar difficulties are experienced with the chicken sarcoma.[2] When first implanted in plasma it grows almost explosively and produces markedly acid foci; but reimplanted it usually fails to grow.

No attempt has been made to determine the nature of the acids

[1] Lambert, R. A., *Jour. Exper. Med.*, 1913, xvii, 499.
[2] Carrel, A., *Jour. Exper. Med.*, 1912, xv, 516.

elaborated during growth *in vitro*. Carbonic and lactic acids are presumably present in greatest amount.

SUMMARY.

Connective tissue cells of chick embryos and cells of a chicken sarcoma, proliferating *in vitro*, soon render acid the plasma about them, but they nevertheless continue to grow well. Evidently the tissue cell will withstand a considerably greater change in the reaction of the fluids about it than has usually been supposed.

Under conditions of *in vitro* life in plasma, which do not provide for an artificial circulation, the acid produced by growing tissues diffuses only slowly and is subject to little dilution from this source. About tissues which grow very rapidly *in vitro*, as, for example, tumor tissues, there must be a marked concentration of metabolic products, and this may largely account for the poor results of attempts at the continuous propagation of such tissues *in vitro*.

EXPLANATION OF PLATE 7.

FIG. 1. Chicken sarcoma in litmus plasma; twenty-four hours' incubation. A pink change indicates tissue growth. The tumor fragments remaining inert are stained blue. Two color photograph; preparation one third larger than natural size.

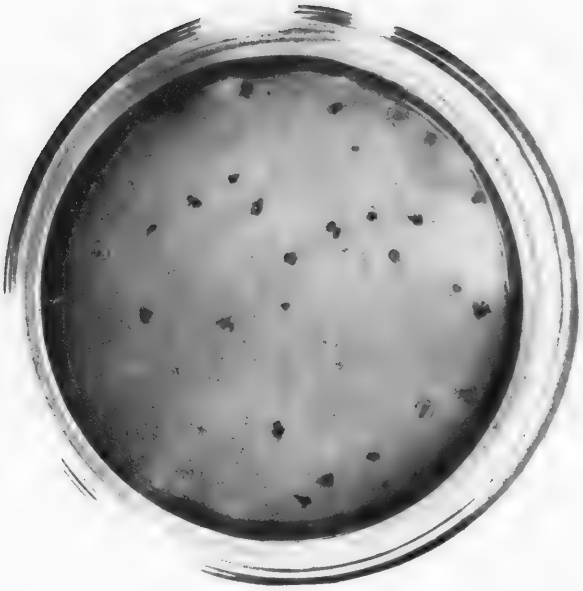

FIG. 1.

(Rous: Growth of Tissue in Acid Media.)

THE SELECTIVE BACTERICIDAL ACTION OF METHYLENE-BLUE.*

By JOHN W. CHURCHMAN, M.D.

(*From the Laboratory of Surgery of Yale University, New Haven.*)

Plate 8.

Since the publication of observations on the selective bactericidal action of gentian violet,[1] studies have been carried on in this laboratory with a large number of other dyes. The technique of divided plates, previously described, has been employed. The results with dyes similar in chemical structure to gentian violet have already been reported.[2] These dyes, like gentian violet, were found to prevent the growth of gentian-positive, and to be without effect on gentian-negative organisms.[3] In this communication the selective bactericidal action of a dye unrelated to gentian violet in chemical structure, namely, methylene-blue, will be reported. That this dye has bactericidal properties has, of course, long been known. It is to the selective feature that I wish to call attention.

Briefly stated, it has been found that methylene-blue, like gentian violet, is without effect on the growth of *Bacillus typhosus* and *Bacillus coli;* but that, unlike gentian violet, it exhibits a selective action when *Bacillus subtilis* and *Micrococcus aureus* are planted on divided plates containing it. The contrast between the action of the two dyes is shown in figures 1 and 2. Figure 1 is a divided plate containing gentian violet and has been stroked with

* Received for publication, June 3, 1913.

[1] Churchman, J. W., *Jour. Exper. Med.*, 1912, xvi, 221; Churchman, J. W., and Michael, W. H., *ibid.*, p. 822.

[2] Churchman, J. W., *loc. cit.*, 1913, xvii, 373.

[3] In previous communications the terms violet-positive and violet-negative were used to describe organisms which, respectively, were or were not restrained by gentian violet. It seems probable that one of the violet dyes (anilin violet, Säureviolet) does not possess the selective action exhibited by gentian violet. Gentian-positive and gentian-negative are therefore more accurate terms.

Bacillus coli, Bacillus subtilis, Micrococcus aureus, and *Bacillus typhosus,* from left to right in the order named. The growth of *Bacillus subtilis* and *Micrococcus aureus* has been restrained; that of *Bacillus typhosus* and *Bacillus coli* has been unaffected. Figure 2 is a methylene-blue divided plate similarly stroked. Here, too, the growth of *Bacillus coli* and *Bacillus typhosus* has been unaffected; *Micrococcus aureus,* which will not grow in the presence of gentian violet, has likewise been restrained by the methylene-blue; but *Bacillus subtilis,* on which gentian violet exercises such a constant restraining effect, has grown on the two sides of the divided methylene-blue plate with nearly equal luxuriance.

In figures 3, 4, and 5 the contrast between the action of gentian violet and methylene-blue is illustrated by means of divided plates containing two central strokes of *Micrococcus aureus,* flanked on either side by a stroke of *Bacillus subtilis.* In figure 3 the upper half of the plate contains gentian violet; and it will be seen that growth of all four strokes stops at some distance from the dividing line. In figures 4 and 5 the upper half of the plate contains methylene-blue; here the growth of *Micrococcus aureus* stops on the proximal side of the dividing line, but *Bacillus subtilis* grows in fair luxuriance in the presence of the dye.

In previous communications I have referred to the precision and constancy of the gentian violet reaction. The same properties cannot be ascribed to the methylene-blue reaction. The selective bactericidal action of the dye is not always sharply manifested; the results of the experiments are not absolutely constant and it is quite simple, by a slight increase in the amount of methylene-blue used in the plate, to prevent the growth of *Bacillus subtilis* as well that of *Micrococcus aureus.* I have attempted to show in figures 4 and 5 that the reaction is a somewhat variable one. In figure 4 the reaction is seen to be sharply selective. In figure 5, on the other hand, there is a trace of growth of *Micrococcus aureus* on the methylene-blue half of the plate, near the dividing line; and the growth of *Bacillus subtilis,* on the methylene-blue agar, is by no means luxuriant.

While there seems to be no doubt that *Micrococcus aureus* is more susceptible to the presence of methylene-blue than is *Bacillus*

FIG. 1.

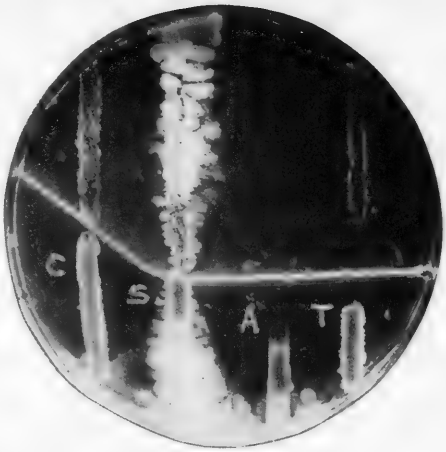

FIG. 2.

FIG. 3.

FIG. 4.

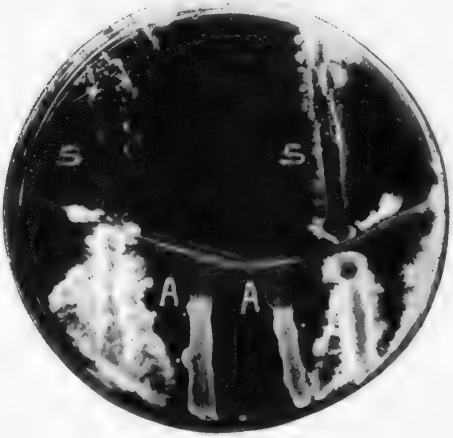

FIG. 5.

(Churchman: Bactericidal Action of Methylene-Blue.)

subtilis, the reservation with which this statement must be made detracts from the importance of the methylene-blue selective reaction. The observations here reported, however, indicate that further studies of other dyes with a similar technique should be carried out.

EXPLANATION OF PLATE 8.

FIG. 1. Gentian violet divided plate which has been stroked with *B. coli, B. subtilis, M. aureus,* and *B. typhosus,* from left to right in the order named. Compare with figure 2.

FIG. 2. Methylene-blue divided plate which has been stroked with *B. coli, B. subtilis, M. aureus,* and *B. typhosus,* from left to right in the order named. Compare with figure 1.

FIG. 3. Gentian violet divided plate, containing two central strokes of *M. aureus,* flanked on either side by a stroke of *B. subtilis.*

FIG. 4. Methylene-blue divided plate, containing two central strokes of *M. aureus,* flanked on either side by a stroke of *B. subtilis.*

FIG. 5. Methylene-blue divided plate, containing two central strokes of *M. aureus,* flanked on either side by a stroke of *B. subtilis.*

THE RELATION OF THE RATE OF ABSORPTION OF ADRENALIN TO ITS GLYCOSURIC AND DIURETIC EFFECTS.*

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INTRODUCTION.

Deep or intramuscular injections have been practised by physicians for many years. This was not done, however, with the expectation that it would favor absorption; on the contrary, some writers (1) state especially, "that the absorption from the intramuscular tissue with its fewer lymphatics is much slower than from the subcutaneous tissues where the lymphatics are abundant." The intramuscular injection was recommended because of the assumption that "deep injections give rise less often to the formation of abscesses." About nine years ago Meltzer and Auer (2) showed experimentally for the first time that the effects of active drugs appear after intramuscular injections earlier and with greater intensity than after subcutaneous injections. The experiments were made with adrenalin, curare, fluorescëin, and morphin. Doses of curare, for instance, which practically had no effect in subcutaneous injections, brought on paralysis and death when injected intramuscularly. In recent experiments by the same authors (3) in which the previous results regarding the blood pressure action of adrenalin were especially studied and confirmed, potassium cyanide was added to the list of substances which act more profoundly by intramuscular injection. They also found that acute anaphylactic shock can be brought on in highly sensitized guinea pigs by injecting one cubic centimeter of horse serum into the lumbar muscles; whereas such a prompt result is never obtained by a subcutaneous

* Received for publication, June 6, 1913.

injection. Eggleston and Hatcher (4) studied recently the seat of the emetic action of apomorphin, and found that for intramuscular injections the minimum dose of this drug is half that which produces a similar effect upon subcutaneous injection. Of practical importance are the experiments of Morgenroth and Levy (5) on the absorption of diphtheria antitoxin. They found that the intramuscular injection of a small dose of antitoxin, together with a fatal dose of toxin, prevented even induration at the seat of injection, while a much larger dose of the antitoxin administered subcutaneously (again together with a fatal dose of toxin) was incapable of preventing the early death of the animals. It seems therefore to be now a well established fact that substances, whether crystalloidal or colloidal, producing definite effects upon the animal body, manifest these effects earlier and with greater intensity when administered by intramuscular than by subcutaneous injection.

The earlier experiments of Meltzer and Auer with adrenalin dealt with three of its known effects: (*a*) rise of blood pressure; (*b*) dilatation of the pupil on the side in which the superior cervical ganglion has been removed; and (*c*) manifest muscular weakness which follows the injection of larger doses of the drug. These three different manifestations appeared in an unmistakable manner quite rapidly after an intramuscular injection of adrenalin, while after subcutaneous injection of a similar dose of that drug, either no effect at all followed, or the effect was comparatively insignificant and appeared late. Adrenalin, however, is capable of producing another quite important effect upon the animal body, namely, the production of glycosuria. Since the discovery of adrenalin glycosuria by Blum (6), the subject has been studied by many investigators. The influence of the place of injection upon the intensity of the glycosuria has also been variously investigated. For dogs it was stated by Herter and Wakeman (7) that intravenous injections give better effects than subcutaneous, and that intraperitoneal injections give the best effects. For the rabbit the glycosuric effects of subcutaneous injections were compared with those of intravenous injections by Ritzmann (8). According to him the glycosuric effect of adrenalin is similar to that of the same drug upon blood pressure; in both cases intravenous injection of the

drug is more effective than subcutaneous. However, among the numerous recent communications on adrenalin glycosuria no mention is made of the glycosuria after intramuscular injection of an adrenal preparation.

The foregoing brief review brings out the following points. It is now quite safely established that active crystalloidal and colloidal substances are pharmacologically more effective when administered by intramuscular than by subcutaneous injections. This fact has been established especially for the several effects produced by adrenalin when injected into the animal body. The glycosuric action of drugs, however, has not been studied as yet by the method of intramuscular injection. This gap we have tried to fill in the present investigation. We have studied the effect of intramuscular as compared with the subcutaneous injection of adrenalin upon the production of glycosuria.

EXPERIMENTAL DATA.

Method.—The experiments were performed on rabbits. There were forty-nine experiments with two rabbits for each, one of which received the adrenalin intramuscularly and the other subcutaneously. The intramuscular injections were given in the lumbar muscles since, as Auer and Meltzer (9) have shown for rabbits, the absorption is better from these muscles than from any other mass of muscular tissue; the subcutaneous injections were made in one of the lower quadrants of the abdomen. The adrenalin[1] was employed in the usual solution of 1:1,000. Many of the rabbits were not fed for twenty-four hours previous to the experiment. Most of the animals received, immediately before the injection of adrenalin, 100 or 150 cubic centimeters of water through a stomach tube. None of the rabbits were fed during the time of observation after the administration of adrenalin, which usually was twenty-four hours. During the observation the animals were kept in metabolism cages, the bladder being emptied at the beginning and at the end of the period of observation. No experiment was considered as finished until the urine was sugar-free. The urines were always preserved with toluene. The analysis for sugar was made by the Pavy-

[1] The adrenalin employed was prepared by Parke, Davis and Company.

Ramsden method,[2] the reduction being calculated as dextrose. Although rabbit urine always contains some substance which slightly reduces the ammoniacal copper solution, the inaccuracy resulting from it is too insignificant to impair the results in any degree, since our object was not to ascertain the absolute amount of reducing substances present in the urine, but merely a comparison of the amounts of these substances brought about by two different methods of administration of adrenalin.

Results.—As mentioned before, ninety-eight rabbits were injected with adrenalin, one half intramuscularly and the other half subcutaneously. The injections were made in pairs; that is, in each experiment two rabbits were employed at the same time and under

TABLE I.

Adrenalin, 1.0 Cubic Centimeter; Water per Os, 100 Cubic Centimeters. All Animals Fasted Twenty-Four Hours Previous to Injection.

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 109	1,430	68	1.48	24	A 110	1,440	160	1.43	24
A 111	1,480	174	1.10	24	A 112	1,555	160	0.34	24
A 113	2,085	143	0.35	24	A 114	2,110	131	1.38	24
A 115	2,310	104	0.13	24	A 116	2,325	>167	1.18	24
A 117	——	——	——	Died	A 118	1,435	109	2.02	24
A 119	1,840	101	2.85	24	A 120	1,520	88	1.77	24
A 121	1,425	53	0.00	24					
A 123	1,085	58	0.00	24	A 124	1,020	129	0.64	24
A 125	1,390	98	0.83	24	A 126	1,340	147	0.65	24
A 127	1,290	137	1.09	24	A 128	1,225	>179	>3.07	24
A 129	1,430	120	0.76	24	A 130	1,350	139	1.98	24
Average		106	0.86		Average		141	1.45	

the same conditions, each being injected with a like quantity of adrenalin, the one receiving it intramuscularly and the other subcutaneously. The two sets of results are therefore comparable. Different doses of adrenalin were tested, one cubic centimeter of a 1:1,000 solution being the highest and 0.3 about the lowest dose which was systematically tested. The forty-nine experiments consist therefore actually of several series of experiments, which run as fol-

[2] Vernon, H. M., *Jour. Physiol.*, 1902, xxviii, 164.

lows. Ten pairs of rabbits received 1 cubic centimeter per animal, seven pairs received 0.7 of a cubic centimeter, ten pairs received 0.5, eight pairs received about 0.4, and eight pairs received 0.3 of a cubic

TABLE II.

Adrenalin, 0.7 to 0.8 of a Cubic Centimeter; Water per Os, 100 Cubic Centimeters. All Animals Fasted Twenty-Four Hours Previous to Injection.

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 73	1,820	100	1.15	24	A 74	2,060	132	0.99	24
A 75	2,220	149	1.53	24	A 76	2,410	102	1.13	30
A 77	1,070	103	1.00	48	A 78	1,160	96	0.75	48
A 79	1,530	90	0.77	24	A 80	1,550	121	1.76	24
A 85	1,920	111	0.00	24	A 82	1,360	124	1.02	24
A 89	1,160	48	0.00	24	A 84	1,580	175	1.10	24
Average		100	0.74		Average		125	1.13	

centimeter. In addition there were six pairs in which the injected dose varied from 0.7 to 0.8 of a cubic centimeter per animal. The results are given in detail in tables I to VI, each table containing

TABLE III.

Adrenalin, 0.7 of a Cubic Centimeter; Water per Os, 150 Cubic Centimeters. Animals Not Previously Fasted.

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 29	2,170	176	1.05	24	A 30	2,140	145	1.33	24
A 31	1,750	175	0.36	49	A 32	2,170	179	1.74	49
A 33	1,595	171	0.83	48	A 34	1,860	203	1.80	48
A 35	1,550	49	0.00 (Trace?)	24	A 36	1,500	206	3.08	24
A 37	2,170	222	2.69	48	A 40	1,990	276	1.96	48
A 41	1,820	176	0.21	48	A 42	1,720	228+	0.00 (Trace?)	48
A 43	2,920	282	2.23	48	A 44	2,890	265	3.01	48
Average		179	1.06		Average		215	1.86	

the data obtained with a given dose of adrenalin, and all six tables are summarized in table VII. In every case the results of two injec-

tions which are placed opposite one another in the tables were done at the same time.

TABLE IV.

Adrenalin, 0.5 of a Cubic Centimeter; Water per Os, 100 to 150 Cubic Centimeters.

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 45[3]	1,610	196	1.44	24	A 46[3]	1,540	198	3.62	24
A 47[3]	1,820	74	0.00	24	A 48[3]	1,640	114	0.27	24
A 89[4]	1,300	151	0.34	24	A 90[4]	1,310	181	0.75	24
A 91[4]	1,400	154	0.38	24	A 92[4]	1,410	160	1.01	24
A 93[4]	1,750	87	1.16	24	A 94[4]	1,800	193	0.33	24
A 95[4]	2,185	63	0.34	24	A 96[4]	2,290	184	0.45	24
A 105[4]	1,500	157	0.26	24	A 106[4]	1,555	165	1.09	24
A 107[4]	1,630	150	0.80	24	A 108[4]	1,675	213	2.57	24
A 101[4]	1,175	89	0.00	24	A 102[4]	1,300	165	0.00	24
A 103[4]	1,415	103	1.44	24	A 104[4]	1,550	124	1.02	24
Average		122	0.62		Average		170	1.11	

On glancing through the various tables, we are confronted, in the first place, with a discouraging element; the method does not lend itself to the obtaining of uniform quantitative results. Under ap-

TABLE V.

Adrenalin, 0.4 of a Cubic Centimeter; Water per Os, 100 Cubic Centimeters. Animals Not Previously Fasted.

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 49[5]	2,070	89	0.00	24	A 50[5]	1,970	136	0.88	24
A 53	1,630	148	0.87	24	A 54	1,395	159	0.00	24
A 55	1,750	104	0.62	24	A 56	1,670	182	0.43	24
A 63	1,500	101	0.48	24	A 64	1,380	141	2.30	24
A 65	1,670	103	0.00	24	A 66	1,510	102	0.84	24
A 67	1,820	171	2.46	24	A 68	1,720	154	1.44	24
A 69[6]	1,530	117	0.00	24	A 70	1,360	102	1.73	24
A 71[7]	1,250	87	0.24	24	A 72[8]	1,220	146	0.13	24
Average		115	0.58		Average		140	0.97	

[3] Not previously fasted. Received 150 c.c. of water *per os.*

[4] Fasted twenty-four hours before experiment. Received 100 c.c. of water *per os.*

[5] Received 150 c.c. of water *per os.*

[6] Adrenalin, 0.45 c.c.

[7] Adrenalin, 0.38 c.c.

[8] Adrenalin, 0.37 c.c.

TABLE VI.

Adrenalin, 0.3 of a Cubic Centimeter; Water per Os, 100 Cubic Centimeters.

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 51[9]	1,270	112	0.00	24	A 52[9]	1,080	155	1.88	24
A 57[9]	1,650	87	0.43	24	A 58[9]	1,600	90	0.79	24
A 59[9]	1,820	69	0.16	24	A 60[9]	1,700	89	0.00	24
A 61[9]	2,270	122	2.32	24	A 62[9]	1,940	94	0.20	24
A 97[10]	1,400	72	0.00	24	A 98[10]	1,505	149	0.54	24
A 99[10]	1,525	86	0.00	24	A 100[10]	1,525	162	0.11	24
A 131[10]	1,490	99	0.17	24	A 132[10]	1,480	118	0.12	24
A 133[10]	2,070	161	0.29	24	A 134[10]	2,050	106	0.27	24
Average		101	0.42		Average		122	0.49	

TABLE VII.

Summary of Tables I to VI.

	Manner of injection.	No. in which no glycosuria occurred.	Urine in c.c.	Dextrose in urine in gm.
Table I. 1.0 c.c. adrenalin	Intramuscular	2	106	0.86
Fasted—10 pairs	Subcutaneous	0	141	1.45
Table II. 0.7–0.8 c.c. adrenalin	Intramuscular	2	100	0.74
Fasted—6 pairs	Subcutaneous	0	125	1.13
Table III. 0.7 c.c. adrenalin	Intramuscular	1	179	1.05
Not fasted—7 pairs	Subcutaneous	1	215	1.85
Tables II and III. 0.7–0.8 c.c. adrenalin	Intramuscular	3	142	0.91
13 pairs	Subcutaneous	1	172	1.52
Table IV. 0.5 c.c. adrenalin	Intramuscular	2	122	0.62
2 pairs not fasted, 8 pairs fasted —10 pairs	Subcutaneous	1	170	1.11
Table V. 0.4 c.c. adrenalin	Intramuscular	3	115	0.58
Not fasted—8 pairs	Subcutaneous	1	140	0.97
Table VI. 0.3 c.c. adrenalin	Intramuscular	3	101	0.42
4 pairs fasted, 4 pairs not fasted —8 pairs	Subcutaneous	1	122	0.49
Tables I to VI. 0.3–1.0 c.c. adrenalin.	Intramuscular	13	120	0.73
All experiments—49 pairs	Subcutaneous	4	152	1.20

[9] Not previously fasted.

[10] Fasted twenty-four hours previous to experiment.

parently similar conditions and by the same mode of administration a given dose of adrenalin gave rise to very variable quantities of dextrose in the urine. This held true for both modes of injection. Under these circumstances we might readily expect that in one experiment it would be an intramuscular injection of adrenalin, which would give rise to a larger quantity of dextrose in the urine, while in another experiment the larger quantity of dextrose would follow a subcutaneous injection. Evidently a small number of experiments would not permit one to draw any definite conclusions. However, the number of experiments is great enough to bring out the differences produced by the two modes of injection even under these discouraging circumstances. Hence an analysis of the various data given in the tables shows unmistakably that subcutaneous injections of adrenalin give rise more often to the appearance of large quantities of dextrose in the urine than do intramuscular injections,—a conclusion quite opposite to that which we had reason to expect. When we take, in the first place, the amount of sugar eliminated by all the forty-nine animals of each set, we find that the average amount for each animal is 1.2 grams for the subcutaneous and 0.73 of a gram for the intramuscular injections. *The glycosuria produced by intramuscular injection is less than two thirds of that produced by the same dose of adrenalin administered subcutaneously.* The difference in favor of the subcutaneous injection remains constant for every dose of adrenalin tested. But with such a small, practically minimum dose, as 0.3 of a cubic centimeter per animal (table VI), the difference in favor of the subcutaneous injection is so small as to be within the limits of experimental error.

The same holds true in general when the dose of adrenalin administered is calculated per kilo of body weight of the animal (table VIII). Here again the average amount of dextrose appearing in the urine after an intramuscular injection of adrenalin is tangibly less than that which follows a subcutaneous injection. The difference in favor of the subcutaneous injection is manifest for every dose of adrenalin used, except for doses below 0.2 of a cubic centimeter per kilo of body weight.

Another instructive fact is the number of failures occurring with both modes of administration. With each mode of administration

there were rabbits which did not react with a glycosuria after an injection of adrenalin; but out of the forty-nine intramuscular injections there were thirteen failures, while of the same number of

TABLE VIII.

Summary of Results with Dose Calculated to Body Weight.

Adrenalin in c.c. per kilo.	No. of experiments performed.	Manner of injection.	Urine in c.c.	Dextrose in urine in gm.
0.70 and over	6	Intramuscular	89	0.69
	5	Subcutaneous	141	1.67
0.50–0.69	5	Intramuscular	103	1.23
	7	Subcutaneous	132	1.32
0.40–0.49	8	Intramuscular	118	0.39
	4	Subcutaneous	183	1.43
0.35–0.39	5	Intramuscular	147	0.85
	6	Subcutaneous	186	1.13
0.30–0.34	7	Intramuscular	158	0.93
	10	Subcutaneous	149	1.49
0.25–0.29	4	Intramuscular	103	0.63
	5	Subcutaneous	150	1.07
0.20–0.24	9	Intramuscular	121	0.65
	8	Subcutaneous	169	0.87
Below 0.20	5	Intramuscular	106	0.64
	4	Subcutaneous	97	0.32

subcutaneous injections there were only four failures. Whatever the factor may be which interferes with the production of glycosuria, it is manifest that it operates more frequently when the adrenalin is administered intramuscularly than when it is administered subcutaneously, which is in harmony with the finding that the glycosuria occurring after a subcutaneous injection of adrenalin is generally greater than after an intramuscular one.

It might be supposed that the diminished averages of the urinary sugar output and the larger number of failures after intramuscular injections of adrenalin are not two separate facts, but that the lower averages after intramuscular injections are merely caused by the greater number of failures, and that the actual glycosurias, when they occur, are as severe as those, or perhaps even more severe than

those produced by subcutaneously administered adrenalin. That this is not the case may be seen from table IX, which gives the actual glycosuria for each dose. In making up the averages, the animals which did not react with glycosuria were excluded from

TABLE IX.

Summary of Actual Glycosurias.

	Manner of injection.	No. in which no glycosuria occurred.	Average excluding the cases having no glycosuria. Urine in c.c.	Average excluding the cases having no glycosuria. Dextrose in gm.
Table I. 1.0 c.c. adrenalin.....	Intramuscular	2	118	1.07
Fasted—10 pairs.....	Subcutaneous	0	141	1.45
Table II. 0.7–0.8 c.c. adrenalin.....	Intramuscular	2	111	1.11
Fasted—6 pairs.....	Subcutaneous	0	125	1.13
Table III. 0.7 c.c. adrenalin.....	Intramuscular	1	200	1.23
Not fasted—7 pairs.....	Subcutaneous	1	212	2.15
Table II and III. 0.7–0.8 c.c. adrenalin	Intramuscular	3	164	1.18
13 pairs.....	Subcutaneous	1	169	1.65
Table IV. 0.5 c.c. adrenalin.....	Intramuscular	2	133	0.77
Not fasted—2 pairs, fasted—8 pairs.....	Subcutaneous	1	170	1.23
Table V. 0.4 c.c. adrenalin.....	Intramuscular	3	120	0.93
Not fasted—8 pairs.....	Subcutaneous	1	138	1.11
Table VI. 0.3 c.c. adrenalin.....	Intramuscular	3	108	0.67
Fasted—4 pairs, not fasted—4 pairs.....	Subcutaneous	1	126	0.56

the calculations. The proportion of the average glycosuria for the subcutaneous and intramuscular injections after this exclusion is 7.63 to 5.78 grams or 100:76. A difference in favor of the subcutaneous injection after exclusion of the failures is present also in the averages made up for each dose tested, except for the smallest dose of 0.3 of a cubic centimeter per kilo of body weight.

The experiments clearly indicate that the chances for the production of glycosuria by adrenalin are greater when it is administered subcutaneously than when given intramuscularly, and that, when glycosuria is produced, the amount of dextrose in the urine is likely to be greater after a subcutaneous injection than after an intramuscular one.

The Volume of Urine after Subcutaneous and Intramuscular Injections of Adrenalin.—All the forty-nine pairs of rabbits received, as stated above, 100, and, in some experiments, 150 cubic centimeters of water, shortly before the injection of adrenalin. Owing probably to this precaution, the diuresis was sufficient for all the rabbits; there were no failures in this respect. The largest quantity of urine passed in twenty-four hours by an individual animal was 276 cubic centimeters (subcutaneous injection), and the smallest quantity amounted to forty-nine cubic centimeters (intramuscular injection); both animals belonged to the group which received 150 cubic centimeters of water and 0.7 of a cubic centimeter of adrenalin (table III). The average quantity of urine for all animals was about 100 cubic centimeters. Here again the average volume of urine of the intramuscular was less than that of the subcutaneous animals, the proportion being 120:152, or 4:5. The proportion for the dextrose was 2:3. The difference in the quantity of urine in favor of the subcutaneous animals is manifest in each of the groups of animals receiving the same dose of adrenalin.

That the variations in the amount of dextrose in the urine were not due to the variations in the quantities of urine passed, is evident from the fact that there were, as mentioned above, among both classes of animals instances in which there was no dextrose in the urine, while some of the quantities of sugar-free urine which these animals passed were: 228, 165, 117, and 112 cubic centimeters in twenty-four hours. On the other hand, we met with instances showing fairly low volumes of urine containing relatively large quantities of dextrose; for instance, 88 cubic centimeters, 1.77 grams; 68 cubic centimeters, 1.48 grams; 87 cubic centimeters, 1.16 grams. While these figures bear witness to the existence of a considerable independence of the glycosuria from the diuresis, in our experiments a survey of all the figures shows that in a general way a certain parallelism prevails between the two phenomena, so that the larger quantities of urine are often accompanied by relatively larger quantities of dextrose, and *vice versa*.

THE EFFECT OF DISTRIBUTION OF A SUBCUTANEOUS DOSE OF ADRENALIN.

In the foregoing experiments the fact has been brought out that in two respects the subcutaneous injection of adrenalin proved to be superior to the intramuscular; it caused a more marked diuresis and favored the appearance of dextrose in the urine. As regards the latter, the amount of dextrose was perceptibly higher, and the number of failures to react with a glycosuria was definitely smaller after subcutaneous than after intramuscular injection. These results are diametrically opposite to those previously obtained by Meltzer and Auer in testing some of the other reactions of adrenalin upon the animal body. Here the effects were unquestionably more favorable after intramuscular injections. In these experiments the favorable action was ascribed to a better absorption of adrenalin from the intramuscular tissue. Could the results of the present series of experiments mean that with regard to the effects upon diuresis and the appearance of dextrose in the urine adrenalin is better absorbed from the subcutaneous than from the muscular tissue? Such a conclusion could be accepted only on the basis of an assumption that adrenalin consists of a complex of substances; that, for instance, the action upon the blood vessels emanates from one substance which is better absorbed from the muscle tissue, and that glycosuria is produced by another component of adrenalin which is better absorbed from the subcutaneous tissue,—an assumption not readily acceptable. Or could it be that the favorable effect of the subcutaneous injections in the experiments was due to the fact that absorption from the subcutaneous tissue is less rapid? Were this the case, then any factor which favors absorption in general ought to prove an unfavorable element in the production of glycosuria by adrenalin. The last mentioned point has been tested by us in the following manner. Meltzer (10) showed some years ago that the distribution of a substance over several areas of the body increases its effectiveness. A dose of strychnin, for instance, becomes more effective when injected into several places. We have therefore tested the influence of the distribution of adrenalin over several places upon the production of glycosuria and

diuresis. This series comprised twenty rabbits, of which twelve received 0.7 of a cubic centimeter of adrenalin as a dose, six received 0.75, and two received 0.8 of a cubic centimeter. The adrenalin was given subcutaneously and each dose was distributed over four places. Each animal received 100 cubic centimeters of water previous to the injections of adrenalin. The detailed results are given in table X. Table II contains the results of

TABLE X.

Subcutaneous Distribution of Adrenalin.

Adrenalin, 0.7 to 0.8 of a Cubic Centimeter, Distributed in Four Places Subcutaneously; Water per Os, 100 Cubic Centimeters.

All Animals Previously Fasted for Twenty-Four Hours.

No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 142[11]	1,160	105	0.00	24
A 143[11]	1,430	118	>0.96	24
A 144[11]	1,390	90	0.00	24
A 145[11]	1,240	89	0.00	24
A 146[11]	1,980	117	0.37	24
A 147[11]	2,100	146	0.00	24
A 148[11, 12]	1,860	59	0.60	24
A 149[11]	1,890	51	0.34	24
A 150[13]	1,900	141	0.00	24
A 151[13]	1,690	151	1.12	24
A 152[11]	1,620	106	1.05	24
A 153[14]	2,260	134	2.65	24
A 154[14]	2,010	80	2.09	24
A 155[13]	1,780	145	1.05	24
A 156[11]	1,310	73	0.00	24
A 157[13]	1,620	69	0.35	24
A 158[13]	1,780	104	1.06	24
A 159[11]	1,380	96	0.47	24
A 160[11]	1,570	88	0.00	24
A 161[13]	1,680	45	0.28	24

Average of all, 100 c.c. urine, 0.62 gm. dextrose.
Average of 13 glycosurias, 98 c.c. urine, 0.95 gm. dextrose.

similar doses injected into one place. There is a clear difference in the results of the two tables, and the difference is definitely in favor of the single injections. While there were no failures in the subcutaneous injections in table II (and only one failure in table III with single subcutaneous injections of 0.7 of a cubic centimeter),

[11] Adrenalin, 0.7 c.c.
[12] Pregnant.
[13] Adrenalin, 0.75 c.c.
[14] Adrenalin, 0.8 c.c.

we find seven failures in table X; that is, seven rabbits out of twenty failed to show glycosuria after receiving subcutaneously a dose of 0.7 to 0.8 of a cubic centimeter injected into four different parts. Furthermore the average amount of dextrose in the urine after single injections (table II) was 1.13 grams, while the average amount of dextrose for the twenty rabbits in table X was only 0.62 of a gram; and even after deducting the seven rabbits with sugar-free urine, the average amount of dextrose for the remaining thirteen rabbits was only 0.95 of a gram. Finally, the average volume of urine for twenty-four hours, when the adrenalin was given subcutaneously in a single spot, amounted to 125 cubic centimeters, while the average amount of urine for the twenty animals which received their dose of adrenalin subcutaneously, distributed over four areas (table X), was only about 100 cubic centimeters. Evidently the distribution of a dose of adrenalin has the tendency to diminish the quantity of urine voided in twenty-four hours, to increase the number of cases in which no dextrose at all appears in the urine, and, when glycosuria is produced, to decrease the quantity of dextrose in the urine.

If we assume that the above mentioned observation of Meltzer holds good also for adrenalin, namely, that the distribution of an injected substance over several areas favors its absorption, we must come to the conclusion that the better the absorption of adrenalin the less effective are its glycosuric and diuretic actions.

DISCUSSION.

The starting point for our investigation was the earlier experiments of Meltzer and Auer with adrenalin. For three of the actions of that drug, the raising of the blood pressure, the dilatation of the pupil, and the muscular weakness, the intramuscular injections are more effective than the subcutaneous. Naturally it was anticipated that a similar effect would be found with respect to the glycosuric action of adrenalin. What was obtained was a diametrically opposite result, that is, glycosuria was brought on with greater certainty and, in most instances, with greater intensity by subcutaneous than by intramuscular injection.

The glycosuric action of adrenalin differs from some of its other known

actions in several respects. In the first place, there is the proportion between the injected quantity and the extent of the effect. For the hemodynamic principle it has been shown (11) that under proper conditions the same quantity of the drug can be relied upon to bring about the same degree of effect. The same seems to hold good to a considerable degree also for the dilatation of the pupils, as was recently shown by Joseph (12). Regarding the glycosuric effect Herter (13) stated that "within certain limits the quantity of the sugar excreted appears proportional to the dose of adrenalin." It seems that this is indeed true only "within certain limits." Herter himself says soon after, that "there is reason to believe that when a certain dose is followed by a large excretion of sugar, a larger dose would not have materially increased this output." In our experiments the average amount of dextrose in the urine decreased in general with the decrease of the dose injected, subcutaneously as well as intramuscularly. This is especially the case for the doses of 0.7, 0.5, 0.4, and 0.3 of a cubic centimeter for an animal. At the same time the individual amounts of the urinary dextrose varied considerably for each dose, so that in some instances the dose of only 0.3 has given rise to the presence of a quantity of sugar in the urine which was larger than that of other cases after an injection of 0.7 of a cubic centimeter. Judging by the figures in various protocols, similar conditions apparently prevailed in the experience of other investigators.

We may state here in passing that some of the discrepancies can perhaps be accounted for by the circumstances that the subcutaneous injections of some investigators may have been actually intramuscular, as it may readily happen that inadvertently the point of the needle instead of remaining in the subcutaneous tissue penetrated the muscular fascia and was therefore actually within a muscle. On the other hand, it may also happen that a needle passed through a muscle bundle and the injection was actually subcutaneous (that is, the injected fluid passed into the loose connective tissue), instead of being intramuscular. It was for the purpose of eliminating such errors that in our experiments the solid mass of the lumbar muscles was selected for the intramuscular, and the lower quadrants of the abdominal wall with its loose skin for the subcutaneous injection. However, occasionally such an error may have happened to us, and may perhaps account for one or the other case with a high intramuscular and a low subcutaneous glycosuria.

At any rate, it is evident that the exactness of the relation of adrenalin to glycosuria falls far behind that of the relation of adrenalin to its effects upon blood pressure.

Another striking difference is to be found in the length of the interval between the administration of the adrenalin and the appearance of the effect. The comparatively slow onset of the glycosuria can in no way be compared with the promptness of the rise of blood pressure following an injection of adrenalin or the promptness of the appearance of the dilatation of the pupil after the removal of the superior cervical ganglion.

A further noteworthy difference is the disappearance of the glycosuric effect after repeated injections of adrenalin and the persistence of the effect upon the blood pressure and upon the dilatation of the pupil. This disappearance after repeated injections has been observed and studied by Pollak (17) and others. The persistence of the blood pressure effect is a well known fact noted by various

writers. The persistence of the pupillary reaction to adrenalin has been established by numerous observations in this laboratory. Ganglionectomized rabbits received injections of adrenalin frequently for weeks and months, sometimes every day and sometimes even several times on the same day, without the pupil ever failing to react promptly, and practically with the original intensity.

Evidently the production of glycosuria by adrenalin is a more complicated process than is the rise of blood pressure or the dilatation of the pupil and it is more difficult to control the numerous factors involved.

In view of the foregoing, it should occasion no surprise to find a difference between the glycosuric effect of adrenalin and some of its other effects. We should have found occasion for surprise if we had been compelled to interpret the difference to mean that for the production of glycosuria adrenalin is more readily absorbed from the subcutaneous than from the intramuscular tissue, while with regard to its other effects adrenalin is better absorbed from the muscles. Such an interpretation is, indeed, not indispensable. As we have seen above, our results permit us to assume that even for the production of glycosuria, adrenalin enters the circulation with greater readiness from the muscle than from the subcutaneous tissue. For the understanding of the results we need only to assume further that the production of glycosuria is rather favored by a slow entrance of the injected adrenalin into the circulation. This assumption finds support in the fact that the distribution of the injected dose of adrenalin over several areas, a procedure which favors a more rapid entrance of the chemical into the circulation, tangibly impairs the production of glycosuria. With this as a basis there is no difference between the experience of Meltzer and Auer with regard to the other effects of adrenalin and the present results with reference to its glycosuric action; in both cases adrenalin is better absorbed from the muscular than from the subcutaneous tissues.

The difference which our experiments show to exist between the glycosuric effect and the other effects of adrenalin consists, then, if our view is correct, in the fact that, for instance, the hemodynamic and the pupillodilating actions of adrenalin are the more effective the more rapidly the adrenalin gets into the circulation, while the glycosuric action, on the contrary, manifestly becomes greater with the retardation of the absorption of the drug into the blood.

Is this view probable, and is there any other evidence in favor of it? In the experiments of Meltzer and Auer with the hemodynamic action of adrenalin, it was found that an intramuscular injection of adrenalin acts nearly as well as an intravenous administration. Is a subcutaneous injection of adrenalin more favorable to the production of glycosuria than an intravenous one? The above mentioned and frequently quoted investigations of Ritzmann (14) seem to speak against such a conclusion. Adopting the method of Kretschmer (15) which the latter used for the study of the blood pressure-raising effect of adrenalin, Ritzmann infused intravenously very dilute solutions of adrenalin and found that not only does this method produce glycosuria, but the amount of dextrose in the urine produced by it exceeds considerably the amount of dextrose in the urine produced by a subcutaneous injection of a much larger dose of adrenalin. Underhill (16) who repeated Ritzmann's experiments found that the intravenous infusion of adrenalin in unanesthetized rabbits causes no glycosuria at all, and that the glycosuria observed by Ritzmann was produced by the simultaneous use of urethane as an anesthetic. Underhill further noted that a subcutaneous injection of adrenalin is more likely to cause glycosuria than an intravenous dose of the same size. Pollak (17) stated that the experience of the pharmacological laboratory of the University of Vienna is that a single intravenous injection of adrenalin, in doses ranging from 0.1 to 1 milligram, does not produce glycosuria, while, as we have seen, some of these doses readily produce glycosuria by subcutaneous injection.

Hence, our conclusion that the favorable effect which we obtained by subcutaneous injection of adrenalin was due to the slow absorption, and that any means which promotes absorption may impair the production of glycosuria finds, therefore, ample support in the statements of Underhill and of Pollak. Moreover it is not contradicted by the findings of Ritzmann which were influenced, as is shown by Underhill, by the use of urethane.

The many failures in our experiments to produce a glycosuria after the intramuscular injections and the still larger number of failures from the subcutaneous injections distributed over several

areas find their ready interpretation in the view that in both instances the absorption into the circulation was so rapid as to imitate an intravenous injection.

Pollak found that intravenous injections, which fail to cause glycosuria, nevertheless cause hyperglycemia, the degree of the latter being insufficient to produce glycosuria in the absence of increased diuresis. He observed further that the hyperglycemia produced by the subcutaneous injection of a certain dose of adrenalin exceeds that caused by a similar dose administered intravenously. Pollak calls attention to the diuretic action of adrenalin and believes that the following rules regulate the relation of glycosuria to hyperglycemia and diuresis. When a hyperglycemia in rabbits is more than 0.15 per cent., but not above 0.25 per cent., and is not accompanied by an increased diuresis there will be no glycosuria. If this moderate hyperglycemia is accompanied by an increased diuresis or if the hyperglycemia exceeds 0.25 per cent., without diuresis, glycosuria results. Our experiments show that subcutaneous injection, which favors glycosuria, also generally favors an increase in the diuresis. At the same time it is quite evident that both processes are independent of each other to a considerable extent.

To the above we wish to add the following remark. While we are sure that a theory which attempts to explain completely the reasons why subcutaneous injections, which have to act through the circulation, are superior to direct intravenous injections, would be premature at present, since our knowledge of the facts in question is still too scant, we nevertheless believe that an analysis of the phenomena should also take into consideration the action of adrenalin upon the process of elimination. Meltzer and Auer (18) previously pointed out that adrenalin restricts absorption as well as elimination, and they indicated that it may interfere with the eliminating power of the kidney. Drummond (19) has shown that adrenalin causes a (reversible) cloudy swelling of the kidney epithelium. Ritzmann (20) stated that when the intravenous infusion of the adrenalin solution took place too rapidly, no sugar appeared in the urine and there was, indeed, little or no urine. When the animal recovered from the effects of such an infusion it passed

some urine which often contained blood. From our personal experience we are confident that larger doses of adrenalin interfere with the elimination of sugar as well as of urine. For this reason we did not employ doses exceeding one milligram of adrenalin. The most satisfactory results relating to the presence of dextrose and to the volume of urine were obtained by us from doses of 0.7 of a cubic centimeter of the adrenalin solution. We believe, therefore, that beyond a certain point the increase of the dose of adrenalin lessens the elimination of the dextrose and restricts the diuresis. The size of the restricting dose is probably not the same for both functions. The influences of adrenalin upon diuresis and upon glycosuria (both the stimulating and restricting influences) are, we think, two essentially independent processes.

Without offering any theory of our own, we wish merely to state our belief that any theory which attempts to explain the phenomena under discussion should take into consideration the influence of adrenalin upon elimination of dextrose, as well as of urine.

SUMMARY.

Subcutaneous injections of adrenalin are, in contrast with its behavior in the production of the other effects of that drug, more favorable to the production of glycosuria in rabbits than intramuscular injections; the failures are fewer and the quantities of dextrose in the urine are generally larger. In general, as regards the stimulation of diuresis by adrenalin, a subcutaneous injection exerts generally a greater effect than an intramuscular one.

Subcutaneous injections of a certain dose of adrenalin distributed over several areas are far less effective than the administration of that dose in a single injection; they fail frequently to produce any glycosuria, the quantity of dextrose in the urine, when present, being less, and the quantity of urine being generally diminished.

Apparently the more slowly the injected adrenalin reaches the blood, the greater is its effect in producing glycosuria and generally, also, the greater its diuretic action.

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A CONTRIBUTION TO THE RELATION BETWEEN PROTEID CLEAVAGE PRODUCTS AND ANAPHYLAXIS.*

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The leading hypothesis among the attempts to explain proteid sensitization, or anaphylaxis, is that of Vaughan.[1] This view was advanced by Vaughan and Wheeler as early as 1907 and has, with more or less marked modifications, received wide acceptance.[2]

His conception may be summarized as follows: The parenteral introduction of a foreign proteid (sensitization) develops in the animal a proteolytic ferment specific for the proteid introduced. This specific ferment remains in the cells of the sensitized animal as a zymogen and is activated by the second injection of the same proteid. The zymogen itself is conceived not as a new substance in the cell but as a more or less permanent alteration of the atomic structure of the cell proteid molecule. A sensitized animal, therefore, is one whose cells are rich in a specific proteolytic zymogen. As Vaughan conceives all proteids to be composed of a toxic and a non-toxic fraction, and as the injection of a foreign proteid into a sensitized animal brings about activation of the specific zymogen, the active ferment thus formed frees the toxic portion of the proteid molecule and these poisonous substances cause the picture of "anaphylactic intoxication." The first injection of the proteid causes no symptoms because the cleavage or digestion of the foreign proteid by the body cells takes place so slowly that at no one time is there a sufficient amount of the toxic proteid group to cause symptoms. Vaughan also holds the view that "each foreign proteid has its predilection tissue in which it is largely deposited, whose cells it especially sensitizes, and where it is disrupted."[3]

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[1] Vaughan, V. C., *Am. Jour. Med. Sc.*, 1913, cxlv, 161.

[2] We shall not enter into a historical review of this matter, nor do we wish to deny that other investigators may have reached similar views independently of Vaughan. It seems to us, however, that Vaughan has the credit of giving the first sharp, clean cut conception, based on experimental evidence, of the rôle which cleavage products may play in proteid sensitization.

[3] Vaughan, V. C., *loc. cit.*, p. 176.

These views have stimulated investigators to the search for proteolytic ferments and proteid cleavage products both in the blood of fully sensitized animals and in animals during the anaphylactic reaction. We shall not enter into a criticism of the many interesting and contradictory results which have been obtained; for these the reader is referred to the works of Abderhalden,[4] Pfeiffer and Mita,[5] Zunz,[6] and Doerr.[7] We shall merely state that no conclusive evidence has yet been obtained that cleavage products of the reinjected proteid produce the symptoms of the anaphylactic reaction.[8]

In the search for proteolytic ferments and cleavage products the investigators limited themselves to one field, the blood. As far as we are aware no one has sought for them in the specific tissues where active anaphylaxis manifests itself by characteristic functional and anatomical changes, a location, therefore, where these substances theoretically must be present. There are two organs especially available for such an investigation, the anaphylactic lung of the guinea pig[9] and the anaphylactic heart of highly sensitized rabbits.[10] In both instances these organs are respectively the immediate primary cause of death, and, therefore, on the basis of the theory briefly outlined above, enough cleavage products of one kind or another must be present in the substance of these organs themselves to produce the characteristic changes which bring about acute death. Immediate examination of these organs after death ought, therefore, to reveal them, provided that cleavage products are present, and that their amount is not beyond the range of the method employed.

These considerations and the fact that an exceedingly delicate

[4] Abderhalden, E., Die Schutzfermente des tierischen Organismus, Berlin, 1912; *Ztschr. f. physiol. Chem.*, 1912, lxxxii, 109.

[5] Pfeiffer, H., and Mita, S., *Ztschr. f. Immunitätsforsch., Orig.*, 1910, vi, 18.

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[8] For a full discussion see Zunz, E., *loc. cit.*, pp. 288–298.

[9] Auer, J., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1909, liii, 458; *Jour. Exper. Med.*, 1910, xii, 151.

[10] Auer, J., *Zentralbl. f. Physiol.*, 1910–11, xxiv, 957; *Jour. Exper. Med.*, 1911, xiv, 476.

method for the detection of proteid cleavage products was available[11] led us to investigate the problem. For convenience we chose as the material for examination the characteristic lungs of guinea pigs that died acutely from serum anaphylaxis.

EXPERIMENTS.

Thirty-seven guinea pigs were used in all. Of these sixteen were normal controls and twenty-one had been sensitized by the subcutaneous injection of 1 c.c. of horse serum. After intervals of 23, 35, 43, 54, and 57 days the sensitized animals were stretched out on an electric pad to prevent loss of heat, were lightly etherized and a cannula was tied into an external jugular vein. After full recovery from the ether, either 0.5 or 0.9 c.c. of warm horse serum[12] was injected into the jugular cannula and the cannula was then washed clear by a following injection of 1.5 c.c. of Ringer or salt solution. The large majority of the sensitized animals showed typical symptoms and died three to six minutes after the intravenous injection. Three did not die acutely, but were killed by severing the neck. The animals were autopsied immediately after cessation of respiration, and the lungs excised. Their appearance was typical in the cases that succumbed acutely: the lungs remained in full inspiratory position on excision, the surfaces and borders were smooth, the color was pale bluish red; no foam was in the trachea; and, as a rule, no hemorrhages were visible. The lungs were then freed from the trachea and the mass of tissue at the hilus, the various lobes cut through a number of times, swiftly washed off with Ringer solution, dried quickly with filter paper, and then examined at once or cooled at 0° C. until they could be used, which was never later than after two hours. The lungs were not allowed to remain for any length of time at room temperature.

The lungs of the three sensitized animals which were killed ten minutes after reinjection of the horse serum were also found to remain distended after excision from the chest, but their distension was not as marked, and their appearance was less firm than that of the other lungs.

The controls were treated in exactly the same manner: they were stretched out on the electric pad, lightly etherized, and a jugular cannula was introduced; after a few minutes 0.9 c.c. of warm horse serum was injected and the cannula washed clear by 1.5 c.c. of Ringer or salt solution. The horse serum caused no noticeable effect in the controls. About five minutes after the injection, the animals were killed by injecting 4 c.c. of air into the jugular vein. Two controls were killed by severing the neck. On immediate autopsy the lungs of all controls collapsed practically fully on excision and formed a small grayish mass which differed strikingly from the anaphylactic lung. Their further preparation was the same as that of the anaphylactic lungs.

[11] Van Slyke, D. D., *Jour. Biol. Chem.*, 1912, xii, 275.

[12] The horse serum employed was about nine months old.

ANALYTICAL METHODS.

Amino Nitrogen.—The lungs were weighed to within 0.01 gm., then coagulated with hot water, minced fine, and further extracted several times with hot water. The extracts were concentrated *in vacuo*, and a small amount of protein, not coagulable by heat, was precipitated with methyl alcohol. The filtrate was then concentrated *in vacuo* to remove the alcohol. The residue was diluted to 10 c.c. in a measuring flask, and portions of 2 c.c. were used for determination of amino nitrogen by Van Slyke's method,[13] a small apparatus being used, which permitted measurement of the nitrogen gas to within 0.01 c.c. (table I).

Total Non-Coagulable Amino Nitrogen (Free NH_2 of Amino Acids, Peptones, Albumoses, etc.).—The hot water extracts were concentrated *in vacuo* to 10 c.c. and used directly for determination of amino nitrogen, without alcohol treatment. The results give the total nitrogen of the α-amino acids, and the end NH_2 of the higher products (table II).

Non-Coagulable Amino Nitrogen after Hydrolysis.—5 c.c. of the above solution were mixed with 5 c.c. of hydrochloric acid (specific gravity 1.18) and heated for twenty-four hours at 100° C. This treatment ensures complete hydrolysis.[14] The acid was removed by concentration *in vacuo* as completely as possible. The residue was taken up in water, to which a slight excess of suspended calcium hydrate was added. The solution was then again concentrated *in vacuo* to remove the ammonia which is always formed on hydrolysis of animal proteins. The residue was brought to 10 c.c., and portions of 2 c.c. were used for amino nitrogen determination. The latter gives about 75 per cent. of the total nitrogen of the non-coagulable proteins and intermediate products (albumoses, peptones, etc.[15] (table II)).

The experimental basis of the methods will be discussed in connection with another article. The results are accurate to within 1 or 2 mg. of amino nitrogen per 100 gm. of fresh tissue. Samples of the same organ from different animals of the same species vary over a much wider range, however, as is shown by the results tabulated. Most of the analyses were made in duplicate.

RESULTS.

The following tables give the results of this investigation. It must again be noted that the analytical data of table I cannot be compared directly with those of table II, because the lung tissues were treated differently before analysis. The data of table I give the amino nitrogen from compounds not precipitated by alcohol; these are chiefly the simple amino acids. The analytical data in table II represent the free amino nitrogen of the compounds not

[13] Van Slyke, D. D., *loc. cit.*

[14] Van Slyke, D. D., *loc. cit.*, p. 295.

[15] The other 25 per cent. belongs to the ammonia and the amino acids which contain part (tryptophane, arginine, histidine) or all of their nitrogen (proline, oxyproline) in other than amino groups.

TABLE I.

Anaphylaxis.

All guinea pigs were sensitized by the subcutaneous injection of 1 c.c. of horse serum. The reinjection dose was always given into the external jugular vein.

No. of animal.	Sex.	Weight.	Length of sensitization.	Reinjection dose.	Death after	Amino nitrogen per 100 gm. of lung tissue.	Remarks.
I 7	F.	360 gm.	23 dys.	0.5 c.c.	6 min.	31 mg.	Not pregnant.
I 8	M.	380 gm.	23 dys.	0.5 c.c.	10 min.	25 mg.	Killed by cutting cervical cord.
I 9	M.	365 gm.	23 dys.	0.9 c.c.	3 min.	27 mg.	
I 10	M.	335 gm.	23 dys.	0.9 c.c.	3 min.	25 mg.	
I 11	M.	405 gm.	23 dys.	0.9 c.c.	10 min.	25 mg.	Killed by cutting cervical cord.
I 12	F.	455 gm.	23 dys.	0.9 c.c.	3 min.	20 mg.	Very early pregnancy.
H 189	M.	520 gm.	43 dys.	0.5 c.c.	14 min.	20 mg.	
H 190	—	470 gm.	43 dys.	0.9 c.c.	3 min.	28 mg.	
I 1	M.	745 gm.	57 dys.	0.5 c.c.	5 min.	Lost	
I 2	F.	535 gm.	57 dys.	0.5 c.c.	5 min.	23 mg.	Not pregnant.
I 3	M.	465 gm.	57 dys.	0.5 c.c.	5 min.	20 mg.	

Total average: 24.4 mg. of amino nitrogen per 100 gm. of lung tissue.

Normal Controls for Table I.

The animals were killed by injecting air into the jugular vein.

No. of animal.	Sex.	Weight.	Dose of serum.	Method of death.	Time of death after serum.	Amino nitrogen per 100 gm. of lung tissue.	Remarks.
a H 192	M.	385 gm.	0.9 c.c.	10 c.c. of air	6 min.	32 mg.	The air was always injected 5 min. after the horse serum.
b H 192	M.	315 gm.	0.9 c.c.	10 c.c. of air	6 min.	25 mg.	
c H 192	F.	375 gm.	0.9 c.c.	10 c.c. of air	6 min.	23 mg.	Not pregnant.
I 4	M.	265 gm.	0.5 c.c.	4 c.c. of air	6 min.	30 mg.	
I 5	F.	290 gm.	0.5 c.c.	2 c.c. of air	6 min.	28 mg.	Very early pregnancy.
I 6	M.	245 gm.	0.5 c.c.	4 c.c. of air	6 min.	36 mg.	

Total average of controls: 29 mg. of amino nitrogen per 100 gm. of lung tissue.

coagulated by heat, and give not only the amino nitrogen of the amino acids but also that of peptones, albumoses, etc. The figures represent all the nitrogen of the simple α-amino acids, 6 to 10 per cent. nitrogen of the albumose, and up to 50 per cent. of that of the simpler intermediate products, such as peptones and peptids. The analytical data in the last column of table II give the amino nitrogen obtained after converting all these intermediate products into amino acids by complete hydrolysis with hydrochloric acid.

A study of the tables shows that the lungs of guinea pigs which died of typical acute anaphylaxis did not yield more amino nitrogen in the three procedures employed than the lungs of normal control animals. Thus in table I ten anaphylactic animals yielded on the average 24.4 milligrams of amino acids per 100 grams of lung tissue, while six control animals gave 29 milligrams for the same amount of tissue; amino acids were therefore surely not more abundant in the anaphylactic than in control lungs. In table II where the free amino nitrogen not only of the amino acids but also of the peptones, albumoses, etc., was determined, we find the average after acute anaphylactic death (ten animals) to be 61.8 milligrams per 100 grams of lung tissue, while controls (ten animals) show 58.5 milligrams for the same amount of tissue; the small difference between the two averages (3 milligrams) is without significance, as it falls within the range of normal variation. When the non-coagulable amino nitrogen after hydrolysis with hydrochloric acid was determined in five anaphylactic and five control lungs the results again showed no significant differences: the average yield of the anaphylactic lungs per 100 grams of tissue was 172.6 milligrams, while the average for the controls was 171.2 milligrams.

Especial attention may be called to the significance of the data obtained after hydrolysis. If there were a difference in the content of albumoses, peptones, etc., in the lungs of the anaphylactic animals as compared with those of the controls, this discrepancy should be increased because the analysis after complete hydrolysis determines not only the free amino acid nitrogen of the albumoses, peptones, etc., but also the amino acid nitrogen of the amino acids formed from the albumoses, peptones, etc., by the process of hydrolysis; much larger quantities of amino acid nitrogen are thus obtained for

TABLE II.

Anaphylaxis.

The animals were prepared as for table I, except that the lungs were treated for analysis in a different way (see text, page 213).

No. of animal.	Sex.	Weight.	Length of sensitization.	Reinjection dose.	Death after	Amino nitrogen not coagulable by heat.		Remarks.
						Without hydrolysis.	After hydrolysis.	
I 19	F.	485 gm.	35 dys.	0.9 c.c.	3 min.	72 mg.		Early pregnancy.
I 20	M.	395 gm.	35 dys.	0.9 c.c.	9 min.	75 mg.		Killed by cutting cervical cord.
I 21	M.	405 gm.	35 dys.	0.9 c.c.	4 min.	63 mg.		
I 22	M.	440 gm.	35 dys.	0.9 c.c.	3 min.	62 mg.		
I 23	M.	440 gm.	35 dys.	0.9 c.c.	6 min.	67 mg.		
I 35	F.	510 gm.	54 dys.	0.9 c.c.	3½ min.	58 mg.	168 mg.	Early pregnancy.
I 36	F.	317 gm.	54 dys.	0.9 c.c.	4 min.	52 mg.	150 mg.	Early pregnancy.
I 37	F.	520 gm.	54 dys.	0.9 c.c.	4 min.	52 mg.	170 mg.	Early pregnancy.
I 38	F.	490 gm.	54 dys.	0.9 c.c.	5 min.	56 mg.	173 mg.	Late pregnancy.
I 39	F.	370 gm.	54 dys.	0.9 c.c.	3½ min.	61 mg.	202 mg.	Early pregnancy.
					Total average	61.8 mg. of free amino nitrogen per 100 gm. of tissue	172.6 mg. amino nitrogen after hydrolysis per 100 gm. of lung tissue	

Normal Controls for Table II.

No. of animal.	Sex.	Weight.	Dose of serum.	Method of death.	Time of death after serum.	Amino nitrogen not coagulable by heat.		Remarks.
						Without hydrolysis.	After hydrolysis.	
I 24	M.	345 gm.	0.9 c.c.	4 c.c. of air in jugular vein	8 min.	58 mg.		
I 25	M.	280 gm.	0.9 c.c.	4 c.c. of air in jugular vein	9 min.	61 mg.		
I 26	M.	254 gm.	0.9 c.c.	Cord cut	8 min.	53 mg.		
I 27	M.	284 gm.	0.9 c.c.	Cord cut	7 min.	52 mg.		
I 28	M.	304 gm.	0.9 c.c.	4 c.c. of air	9 min.	60 mg.		
I 40	M.	385 gm.	0.9 c.c.	4 c.c. of air	8 min.	62 mg.	185 mg.	
I 41	M.	390 gm.	0.9 c.c.	4 c.c. of air	6 min.	61 mg.	163 mg.	
I 42	F.	265 gm.	0.9 c.c.	4 c.c. of air	7½ min.	59 mg.	178 mg.	Not pregnant.
I 43	F.	320 gm.	0.9 c.c.	4 c.c. of air	6 min.	60 mg.	155 mg.	Not pregnant.
I 44	F.	315 gm.	0.9 c.c.	4 c.c. of air	7 min.	59 mg.	175 mg.	Not pregnant.
					Total average	58.5 mg.	171.2 mg.	

determination, and any difference in the content of these substances in the two types of lung should therefore be more easily detectable. As a matter of fact the differences between the averages of the two kinds of lungs were negligible.

The conclusion therefore is fully warranted that the amino acid, peptone, and albumose content of lungs which show undoubted anaphylactic changes is apparently identical with that of normal controls. Thus our results do not support the proteid cleavage theory of anaphylaxis, yet they do not exclude it, because it is conceivable that specifically toxic cleavage products may be formed in such minute amount that they do not appreciably influence the total quantity of cleavage products normally present.

It may be worthy of notice that fluctuations in the amino nitrogen figures amounting to one third of the amount present may occur in animals which were kept under approximately the same conditions and that neither sex nor pregnancy exerted any definite effect on the content of amino nitrogen in the lung.

SUMMARY.

Examination of the anaphylactic lung by means of Van Slyke's amino nitrogen method gave no evidence of an increased amount of proteid cleavage products of any kind in this organ. This investigation gives no support to the hypothesis that the true anaphylactic lung of the guinea pig is caused by proteid split products.